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DEVELOPMENTAL ANATOMY AND HOMOLOGIES IN WHEAT[1]

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INTRODUCTION

Embryo and seedling morphology and anatomy in various Gramineae, including wheat (*Triticum vulgare* Vill.[3]), have been studied intensively for more than 120 years. It would seem that homologies and other principal features should be definitely settled. However, divergent views presented even in some of the more recent papers dealing with the general subject (Boyd (*3*)[4], Avery (*1*), Howarth (*25*), Percival (*43*) emphasize the fact that observations have not been easily and certainly analyzed.

In 1897, Van Tieghem (*60*), in presenting a complete revision of his first interpretation published 25 years before, stated that "* * * malgré les nombreuses recherches dont il a fait l'objet, c'est encore aujourd'hui, dans l'histoire de ces plantes, l'un des sujets les plus controversés." This statement still applies.

Justification for presenting the present study lies in a hope that at least the observations submitted will help to clarify the situation without further confusing it.

REVIEW OF LITERATURE[5]

The literature of this subject is extensive. It has been well reviewed, however, by Van Tieghem (*59*), Bruns (*4*), Kennedy (*28*), and recently by Avery (*1*), and it seems unnecessary to present here more than a summary of the various conflicting views. Different interpretations of the grass embryo follow:

(1) The scutellum is the cotyledon, the coleoptile being considered the next succeeding leaf, or the first leaf of the plumule, and the epiblast, when present, either of no significance or an appendage of the axis, coleorhiza, or scutellum. This view has been held by Mirbel (*36*), Raspail (*46*), Demoor (*12*), Schacht (*51*), Heiden (*22*), Nobbe

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[3] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum* L.; but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that form.

[4] Reference is made by number (italic) to Literature Cited, p. 318.

[5] A more detailed review of the literature is filed in typewritten form in the library of the U.S. Department of Agriculture, under the title "Review of Literature on the Morphology and Homology of the Grass Embryo", by M. A. McCall.

(*42*), Lermer and Holzner (surmise) (*31*), and Avery (*1*). Malpighi (*35*) is usually considered to have interpreted the scutellum as a seed leaf. It is difficult to tell, however, whether his phrase, "the leaf, which is called the flesh of the seed", refers to the scutellum or to the endosperm and seed coats. Mirbel (*37*) assumed that Gaertner's (*16*) ideas conformed to this view, but Gaertner's writings hardly justify this conclusion. Those supporting the view mentioned above consider that the interval of the axis between the attachment of the scutellum and the divergence of the coleoptile (the mesocotyl of maize) is internode.

(2) The scutellum is the cotyledon; the epiblast, when present, is a rudimentary cotyledon; and the coleoptile is the third leaf of the plant, or the first leaf of the plumule. This view has been held by Poiteau (*45*), A. L. de Jussieu, as reported by Mirbel (*37*), Mirbel (*39*), Turpin (*62*), Kratzmann (*30*), Warming (*63, footnote pp. 446–448*), Hackel (*19*), Bruns (*4*), Van Tieghem (*60*), Coulter (*10*), Weatherwax (*64*), and Percival (*43*). Opinion in this group varies as to the position of the epiblast. Some consider it opposite the scutellum at the same node. Others consider it as belonging to a second node between the scutellum and the coleoptile. This group also interprets the so-called mesocotyl as internode. Van Tieghem (*60*) credited Malpighi (*35*) with believing the epiblast to be a rudimentary cotyledon. There seems to be nothing, however, in Malpighi's (*35*) own statements or in the legends of his drawings to indicate such an opinion on his part. Mirbel (*37*) credits De Jussieu with originating this general theory, and it is on Mirbel's statement that he is placed in this group. Turpin (*62*), a contemporary of A. L. de Jussieu, Mirbel, and Poiteau, on the other hand states definitely that Poiteau (*45*) was the first to interpret the epiblast as a rudimentary cotyledon.

(3) The scutellum and the coleoptile together are the cotyledon, the epiblast, when present, being interpreted as an appendage of the axis, scutellum, or coleorhiza. Mirbel (*38*), Bernhardi (*2*), Schleiden (*52*), Hofmeister (*23*), Hanstein (*20*), Van Tieghem (*59*), Ekkert (*14*), Hegelmaier (*21*), Klebs (*29*), Schlickum (*53*), Celakovský (*7*), Strasburger (*57*), Tannert (*58*), Sargant and Arber (*50*), Worsdell (*65*), Nishimura (*41*), Souèges (*56*), and Howarth (*25*) have held this view. The coleoptile is variously interpreted as ligular, bistipular, or an extension of the cotyledonary sheath. The axis interval between scutellum and coleoptile attachments is interpreted as nodal, as "middle" of the cotyledon, hence the mesocotyl of maize. Hegelmaier (*21*) credited Gaertner (*16*) with first suggesting this opinion, but beyond a possible implication in the legend of one of his illustrations, the writer has been unable to find evidence in Gaertner's writings to confirm this view.

(4) The scutellum and the coleoptile together are the cotyledon, and the epiblast when present is a rudimentary cotyledon. Kennedy (*28*), Cannon (*6*), and Bugnon (*5*) are exponents of this theory. The epiblast apparently is interpreted as a leaf opposite the scutellum at the same node.

(5) The view that the coleoptile is the cotyledon and the scutellum an outgrowth of the radicle or the axis, held by Richard (*47*), Adrien de Jussieu (*27*), Lestiboudois (*32*), Gris (*17*), Hofmeister (*24*), and Sachs (*49*), has not been championed in any important survey of the matter in recent years.

(6) Boyd (*3*) held that the scutellum is an evolutionary fusing of a sucker (as that of *Hedychium*) and a part of the cotyledonary ligule; that the epiblast is a fragment of the sheathing base of the cotyledon and of a part of the cotyledonary ligule; and that the coleoptile is equivalent to the first foliage leaf.

The difficulty of the problem is suggested by the fact that several of those who have studied it have held different views thereon at different times. Mirbel (*36*, *38*, *39*), Hofmeister (*23*, *24*) and Van Tieghem (*59*, *60*) are examples. Holzner (*31*) confessed his inability to arrive at any definite decision. While some one of the several interpretations may have held temporary advantage in general favor at one time or another, none has ever been universally accepted.

MATERIALS AND METHODS

Anatomical studies were made on mature wheat embryos and on seedlings, beginning with early germination. The material used was *Triticum vulgare*, varieties Turkey Red (C.I. no. 1558)[6] and Hard Federation (C.I. no. 4733). These varieties differ widely in morphological and physiological characteristics. Because of these contrasts it was hoped that anatomical features might be so emphasized in one or the other that observations and interpretation would be simplified. The two varieties do not differ essentially, however, in their general anatomy, and, while there are differences in degree, features evident in one are equally clear in the other. Both varieties have been described by Clark, Martin, and Ball (*9*).

Earlier studies [7] showed that, when grown under different temperature conditions, both Turkey and Hard Federation can be considerably modified morphologically. At temperatures of 16° C. and below, both develop the crown at a relatively low level, and all sections of the axis below the crown are comparatively short. At temperatures of 20° and above, the position of the crown is relatively high, and subcrown sections of the axis are correspondingly longer. This lengthening extends even to portions of the axis that normally show little elongation, as for instance the section between scutellum and coleoptile divergences. This is particularly pronounced in Hard Federation plants.

It is apparent that many of the difficulties in interpreting the morphology of the wheat embryo and of the structures arising from it are due to the normally compressed and restricted longitudinal extent of the embryo axis below the plumule and of those sections of the plant axis formed directly from this part of the embryo axis. Any lengthening or extension in this region during germination therefore should be helpful. To obtain as much variation in this respect as possible, different lots of material of the two varieties chosen for study were grown in a controlled-temperature greenhouse in sections held at approximately 12°, 16°, 20°, and 24° C., respectively. A smaller quantity of material also was grown in controlled-temperature soil tanks.

Material for detailed anatomical studies was harvested at regular intervals from early germination, fixed in formal-acetic-alcohol, and embedded in paraffin by the usual methods. Sections were cut

[6] C.I. no. refers to accession number of the Division of Cereal Crops and Diseases.

[7] TAYLOR, J. W., and MCCALL, M. A. THE INFLUENCE OF TEMPERATURE AND OTHER FACTORS ON THE MORPHOLOGY OF WHEAT SEEDLINGS. [Unpublished manuscript.]

at 12μ to 18μ, depending on age. It was necessary to soften some of the oldest material in equal parts of concentrated hydrofluoric acid and 70 percent alcohol for 24 hours before embedding it, in order to cut satisfactory sections. Flemming's triple stain was used.

Drawings were prepared by taking a 5- by 7-inch photomicrograph of any desired section, making from this an ordinary bromide enlargement (about 200 diameters), inking-in the enlargement with waterproof india ink, and bleaching out the photograph with a potassium cyanide-iodine solution. This left a black and white line drawing, which, after being washed and dried, was reduced by photographing. In inking-in the enlargements all doubtful features were confirmed from the original section under the microscope. A similar technic has been described by Naylor (*40*).

The method used by the writer gives illustrations absolutely correct in proportion, and, if carefully checked, as correct in general detail as can be expected from any system involving the human factor (drawing ability, interpretation, etc.). The drawings can be made large enough so that, when they are reduced, irregularities practically disappear. A very decided advantage of the method lies in the fact that the inking-in may be dropped at any stage and taken up again even for a very short interval with no sacrifice in accuracy or necessity of setting up or of constantly readjusting delicate apparatus. Enlargement also may bring out details overlooked when the section was examined under the microscope.

In making drawings by this technic, care must be exercised in using the cyanide-iodine solution, for if it is too strong, the ink as well as the photographic image is removed. The cyanide also is a deadly poison and must be thoroughly removed by careful washing.

OBSERVATIONS

GENERAL MORPHOLOGY

Excellent descriptions of the wheat embryo and of its developmental morphology during germination and early seedling stages are available from several authors. Van Tieghem (*59*), Percival (*43*), and Avery (*1*) all give good accounts. Features are described here only as necessary for identification and later discussion.

The embryo lies in the basal portion of the wheat grain on the dorsal side between the endosperm and the seed coat. Its position is shown in Plate 1, *A*, *a*, *b*. Externally the embryo consists of (1) an axis with a terminal plumule completely enclosed in a tubular cone-shaped sheath, the coleoptile, which is entirely closed except for a vent or slitlike pore in its front face slightly below the tip; (2) a terminal primary root enclosed within a cone-shaped root sheath, the coleorhiza; (3) an oval, fleshy structure, the scutellum (*16*), attached to one side of the axis and making up most of the bulk of the embryo; and (4), opposite to the scutellum, a small scalelike structure, the epiblast (*47*). In all well-developed embryos there is a pair of opposite lateral lobes on the axis in the general region which bears the epiblast. These are the outward evidence of the lateral seminal roots mentioned hereafter. As the embryo lies in the seed, the scutellum is closely appressed to the endosperm, the axis side of the embryo facing the seed coat. A view of the embryo from its front or axis face is shown in plate 1, *B*.

A: *a*, Dorsal view; and *b*, sagittal section through a wheat grain, showing position of embryo. × 10. *B*, Face or axis view of a wheat embryo: *a*, scutellum; *b*, coleoptile; *c*, vent of coleoptile; *d*, epiblast; *e*, lobe caused by lateral root; *f*, coleorhiza; *g*, suspensor. × 30. *C*, Enlarged view of bud in a crown-leaf axil, showing the prophyll, the leaf torn away to expose the bud: *a*, The bud with prophyll; *b*, the leaf base where the leaf has been torn away. × 8.

The embryo is provided with a procambial system (evident either in the side or in the face longitudinal section, figs. 1 and 2), from which, during germination and after, the lower vascular elements of the young-plant axis are developed. There are present, as pointed out above, the primordia of two pairs of lateral roots in the part of the axis between the terminal plumule and the primary root (fig. 2, *d* and *g*). In many but not in all cases there is also a root primordium in the front-face region of the axis back of the epiblast (fig. 3, *B*). Enclosed within the coleoptile are the primordia of the first three foliage leaves and the growing point of the axis. In the axil of the coleoptile, on the same side of the axis as the scutellum, is a bud primordium (fig. 1, *h*).

When the seed is exposed to moisture, water is absorbed and the seed swells. At appropriate temperatures the embryo begins to push out both the coleorhiza and the coleoptile, the former at a somewhat more rapid rate than the latter. The seed coat is ruptured, the coleoptile turning upward, and the primary root piercing the coleorhiza and turning downward. Shortly after the primary root has pierced the coleorhiza the lower pair of lateral roots emerge through the axis cortex, followed soon after by the second pair just above the first. The coleoptile continues to elongate, and the first foliage leaf of the plumule pushes through the vent. The roots increase in length, and the axis above the point of coleoptile attachment grows upward. The second foliage leaf pushes out. In most varieties of wheat sown at a normal depth (1.5 to 2.5 inches), when germination and early growth occur at temperatures of 16° C. and less, elongation of the axis above the coleoptile attachment continues until the node of the first foliage leaf reaches a point varying from 0.5 inch or more below to just at the soil surface. Elongation below this node then ceases. Adventitious roots are formed at this node, which then becomes the first node of the crown. The axis region between the coleoptile attachment and the first crown node is preferably designated the subcrown internode (*34*). Development by 2-day intervals up to 14 days is shown in figure 4.

This takes the young plant nearly to the stage at which the first crown node has reached its final position.

Under usual conditions the crown region of the wheat plant is made up of a variable number of successive nodes and internodes. The latter lengthen very little, and the whole crown structure is much compressed and shortened. At each crown node usually two or more adventitious roots are formed, their position being at almost any point around the nodal periphery. Most commonly, however, the first two roots that appear at a node are located some 90° from the median line of the subtending leaf. These crown roots are the principal supply roots of the plant. They are not exclusively so, however, for, contrary to statements by some authors (*48*), the seminal roots remain alive and functional throughout the life of the plant unless they die from disease or are killed in some other way. Locke and Clark (*33*) noted instances in arid regions in which crown roots did not develop and the root system consisted only of the seminal roots. Simmonds and Sallans (*54*) and Todaro (*61*) have shown that amputation of the seminal roots seriously interferes with subsequent growth and yield.

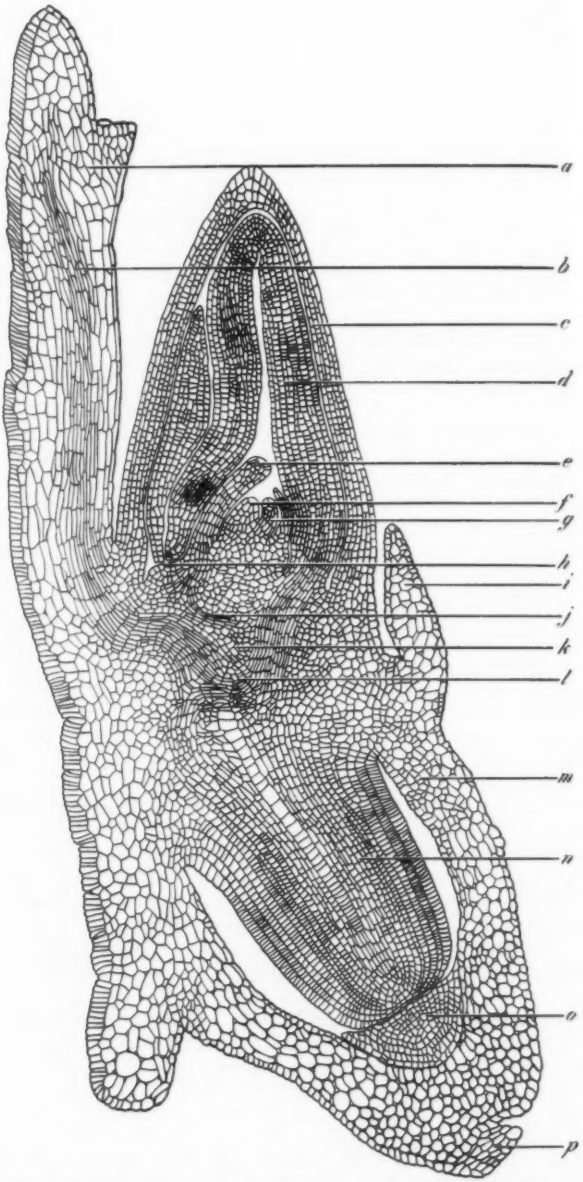


FIGURE 1.—Median sagittal section of a wheat embryo (Turkey variety): *a*, Scutellum; *b*, scutellum trace; *c*, coleoptile; *d*, first foliage leaf; *e*, second foliage leaf; *f*, third foliage leaf; *g*, growing point; *h*, coleoptile axillary bud primordium; *i*, epiblast; *j*, procambium cross-axis plate, second node; *k*, first internode; *l*, first node, transition from root to stem; *m*, coleorhiza; *n*, primary root; *o*, root cap; *p*, remains of suspensor. × 80.

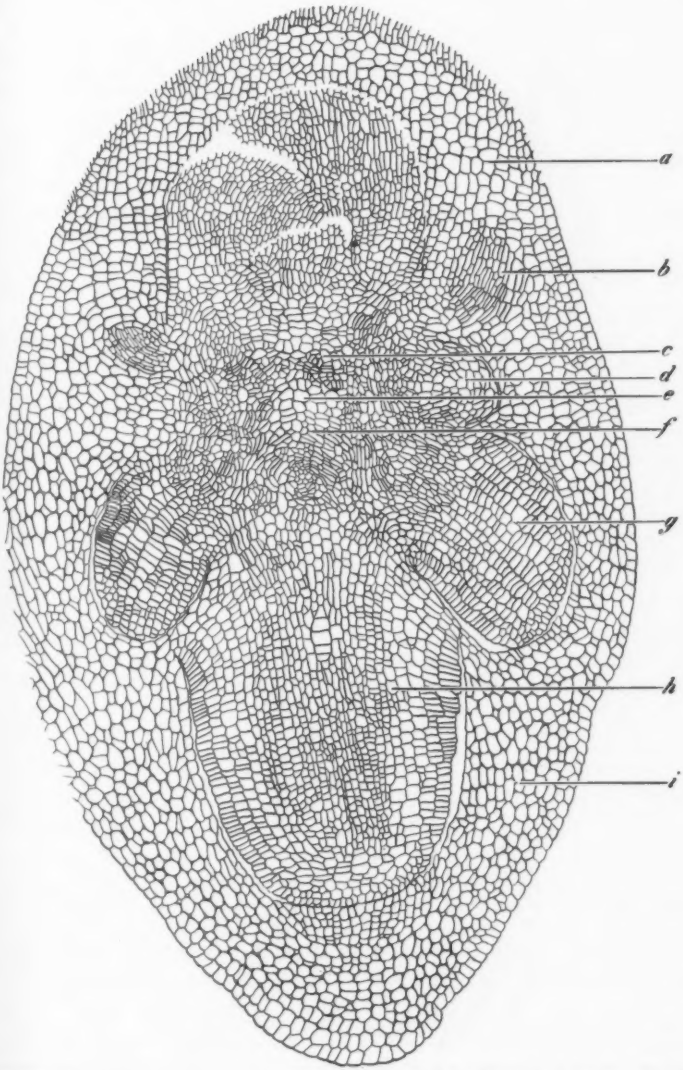


FIGURE 2.—An approximately median face longitudinal section of a wheat embryo (Turkey variety): *a*, Coleoptile; *b*, coleoptile bundle; *c*, procambium cross-axis plate, second node; *d*, root of second lateral pair; *e*, first internode; *f*, procambium cross-axis plate, first node; *g*, root of first lateral pair; *h*, primary root; *i*, coleorhiza. × 80.

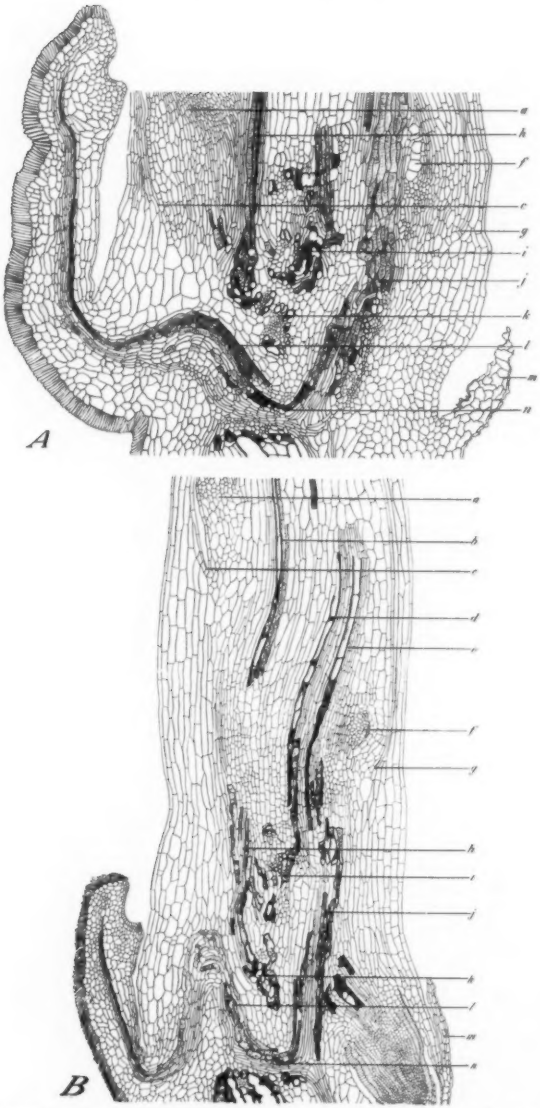


FIGURE 3.—Approximately median sagittal sections through the lower subcrown axis of Hard Federation wheat seedlings: *A*, A seedling with approximately normal elongation in this region, grown at 16° C.: *a*, Coleoptile axillary bud; *c*, posterior coleoptile axis attachment; *f*, tangential vascular connections of coleoptile axillary root; *g*, anterior coleoptile axis attachment; *h*, bundle *2–m* (bundle designations given in figs. 5 to 9, inclusive); *i*, vascular anastomosing region of third node; *j*, bundle *1–m*; *k*, vascular plate of second node; *l*, scutellum trace; *m*, epiblast; *n*, first node, transition of root to stem. ×45. *B*, A seedling with more than normal elongation in this region, grown at 20° C.: *a*, Coleoptile axillary bud; *b*, bundle *1–3l*; *c*, posterior coleoptile axis attachment; *d*, bundle *2–1r*; *e*, bundle *1–1l*; *f*, coleoptile axillary-root primordium; *g*, anterior coleoptile axis attachment; *h*, bundle *2–m*; *i*, vascular anastomosing region of third node; *j*, bundle *1–m*; *k*, vascular plate of second node; *l*, scutellum trace; *m*, epiblast; *n*, firstnode. ×30. This section is at a slight angle to the right and in the upper part does not show bundles *1–m* and *2–m*.

In the leaf axil at each node there is a bud primordium, similar to that in the axil of the coleoptile. These bud primordia develop into secondary axes or tillers, each similar to the primary axis except that its orientation in respect to phyllotaxy, etc., is at 90° to that of the primary axis.

As already noted, during the earlier development of the wheat plant the culm internodes do not elongate. The closely grouped leaves from the crown nodes of the primary axis and of the tillers produce a tuft or rosette, characteristic of the so-called tillering stage in the life history of most grasses. During this stage bud and tiller formation are most active. The present study is not concerned with structures beyond this stage. A good description of developmental morphology for this and later stages is given by Percival (*43*).

The crown is included in this survey only because of certain positional and vascular relationships of leaves, roots, and nodes, and

FIGURE 4.—Relative development of Hard Federation wheat germinated at 16° C., by 2-day intervals to 14 days: *a*, Dry seed; *b*, 2 days; *c*, 4 days; *d*, 6 days; *e*, 8 days; *f*, 10 days; *g*, 12 days; *h*, 14 days. Two fifths natural size.

because of the axillary buds and their prophylls. The prophyll is a characteristic sheathing structure enclosing each axillary bud, and generally interpreted as its first leaf. It is entirely devoid of blade. A bud with its young prophyll is shown in plate 1, *C*. The similarity in appearance of the prophyll and the coleoptile (pl. 1, *B*) is evident. The anatomical features of crown and prophyll that bear on this study will be discussed later.

By the time the first crown node can be identified most of the subcrown structures utilized in the identification of embryo homologies are evident. The exceptions are the adventitious roots which appear just above the divergence of the coleoptile some time after the first crown node has reached its permanent position. There may be only 1 of these roots, or 2, or more rarely 3. Their position varies almost entirely around the axis. The primordia of these roots are not present in the embryo, and therefore they cannot be properly classed as

seminal roots, even though their position so close to the seed and far below the crown might suggest such a designation. As a rule, the growth of these roots is relatively slow, and their primordia may be present for some time before they are externally discernible, which may not be for a month or more after germination begins. In many cases they merely start growth and then make little subsequent

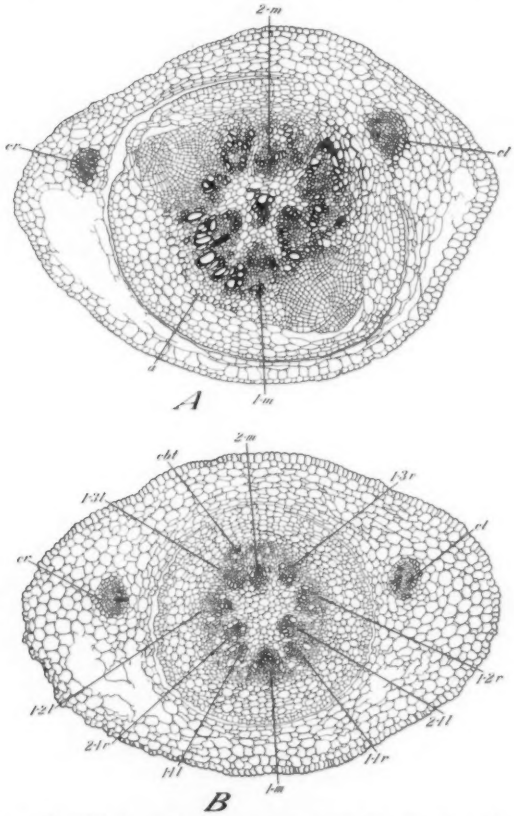


FIGURE 5.—Cross section of the stem axis of wheat seedlings just below complete coleoptile divergence: *A*, a 22-day-old Hard Federation wheat plant grown at 20° C., showing root primordia: *a*, Endodermis; *cl* and *cr*, coleoptile bundles; *1-m*, median bundle of first foliage leaf; *2-m*, median bundle of second foliage leaf. × 44. *B*, a 14-day-old Turkey wheat plant: *cl* and *cr*, Coleoptile bundles; *cbl*, vascular connections of the coleoptile axillary bud; *1-m*, median bundle of first foliage leaf; *1-1l*, *1-2l*, *1-3l*, *1-1r*, *1-2r*, *1-3r*, lateral bundles of first foliage leaf; *2-m*, median bundle of second foliage leaf; *2-1l*, *2-1r*, lateral bundles of second foliage leaf. × 44.

development. The primordia of two roots originating just above the attachment of the coleoptile are shown in cross section in figure 5, *A*.

There may be a maximum of 6 seminal roots in the wheat embryo. However, the primordia of all these are not present in every embryo. In many instances the primordium of a root on the front face of the axis is absent. This was true of the seedling from which figures 5, *B*, to 9, *B*, were drawn. In some cases there may be a primordium for

only one of the second pair of lateral roots (fig. 2). Assuming that there are 3 roots, which may develop above the point of attachment of the coleoptile, a maximum of 9 roots may develop under optimum conditions in the subcrown region of the wheat plant. This, of course, does not apply in cases in which there are several nodes and internodes between the seed and the crown. This latter condition, described by Percival (*43*), is not unusual, but it is not typical. Nor does the usual

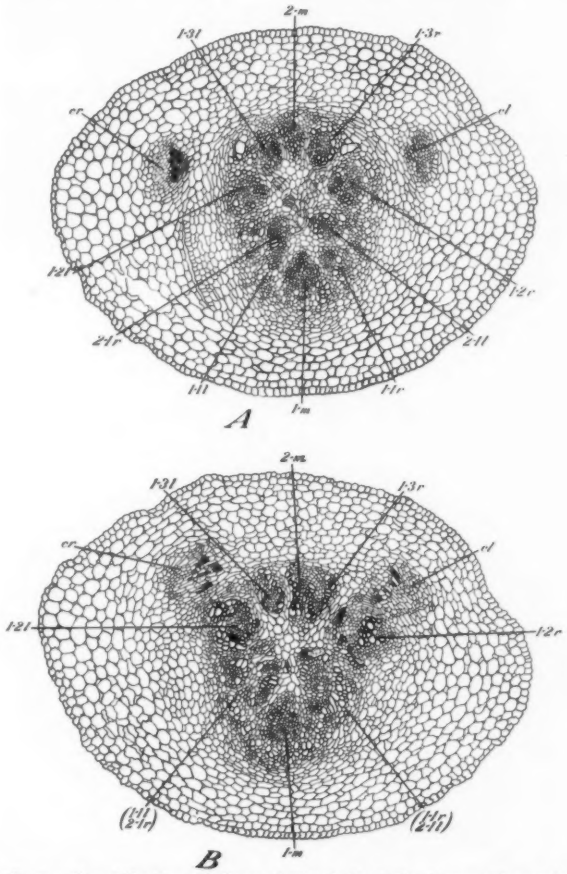


FIGURE 6.—Cross section of the stem axis (same plant as in fig. 5, *B*): *A*, cross-axis vascular connections of region interpreted as third node, just below coleoptile divergence; 0.42 mm lower than figure 5, *B*. × 44. Designations as in figure 5, *B*. *B*, 0.16 mm below figure 6, *A*, in region interpreted as internode. Designations as in figure 5, *B*. × 44.

rule apply when adventitious roots develop on the subcrown internode. Such a condition is rather rare, however, and when it does occur, the roots usually develop in close proximity to a node. Such a root, growing from the subcrown internode just under the first crown node, is shown in figure 10, *A*. The number of subcrown roots that actually

appear is variable, usually ranging from 3 up to the potential maximum of 9. The relation of vascular anatomy to this variability in the number of developing roots will be discussed later.

An enlarged view of the basal region of a seedling wheat plant, showing the different structures discussed above and their positional

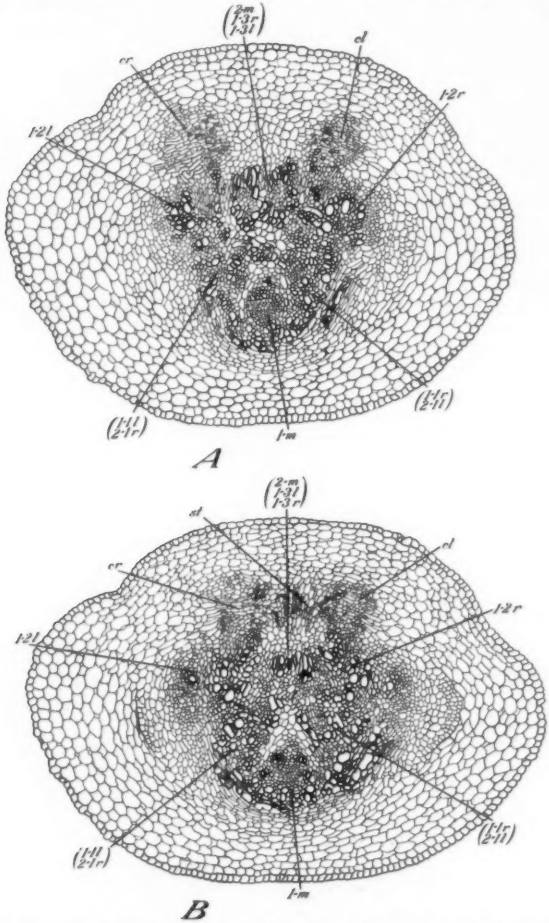
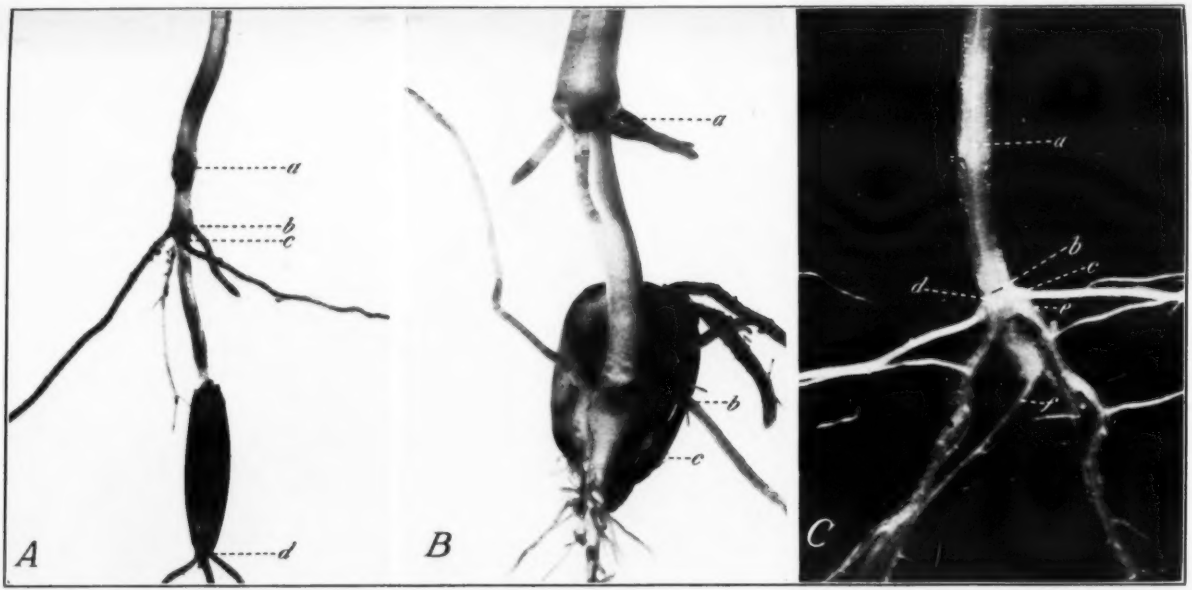


FIGURE 7.—Cross section of the axis (same plant as fig. 5, *B*): *A*, 0.16 mm below figure 6, *B*, in region interpreted as internode, showing divergence of coleoptile bundles to back of axis, vascular connection between coleoptile bundles and stelar bundles, top of divergence of second pair of lateral seminal roots, and less distinct differentiation of stelar bundles. Designations as in figure 5, *B*. × 44. *B*, 0.1 mm below figure 7, *A*, in upper part of second node, showing junction of coleoptile bundles with scutellum trace, connection of coleoptile bundles with stelar bundles, vascular plate of second node, and divergences of second pair of lateral seminal roots and their vascular connections. *sl*, Scutellum trace; other designations as in figure 5, *B*. × 44.

relationships, is shown in plate 2, *C*. The portion of the plant axis below the point of divergence of the coleoptile represents the direct maturing of embryonic structures. Primary attention will be directed to vascular and positional relationships in this region because of their



ENLARGED VIEWS OF THE SUBCROWN REGION OF 14-DAY-OLD SEEDLINGS OF OATS, CORN, AND WHEAT, SHOWING COMPARABLE MORPHOLOGICAL FEATURES.

A, Iogold oat seedling, grown at 16° C.: *a*, Fourth node, crown node; *b*, third node, coleoptile divergence; *c*, second node, scutellum-trace divergence; *d*, first node, transition from root to stem. *B*, Garrick corn seedling, grown at 18° C.: *a*, Third node, coleoptile divergence, crown node; *b*, second node, scutellum-trace divergence; *c*, first node, transition from root to stem. *C*, Turkey wheat seedling, grown at 16° C.: *a*, Fourth node, crown node; *b*, third node, coleoptile divergence; *c*, second node, scutellum-trace divergence; *d*, epiblast; *e*, first node, transition from root to stem; *f*, primary root. The upper black line on the face of the seedling marks coleoptile divergence and the third node, and the lower black line the transition from root to stem or the first node. This region of the axis develops by the direct maturing of embryo tissues. All × 2½.

bearing on embryo interpretation. The position of this region is defined also in plate 2, *C*.

DETAILED ANATOMY

A median sagittal section of an embryo from a mature wheat seed is shown in figure 1. The positional relations of axis, scutellum,

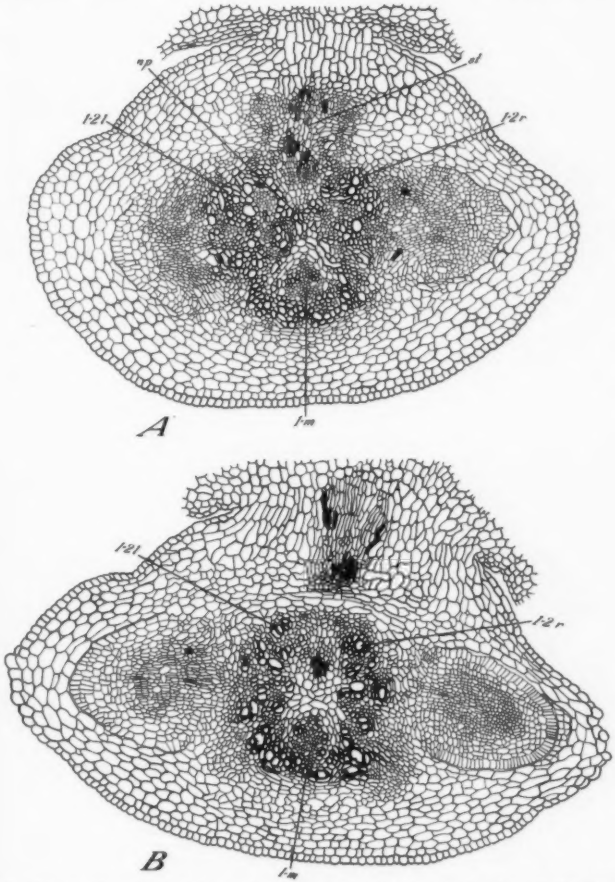


FIGURE 8.—Cross section of the axis (same plant as fig. 5, *B*): *A*, 0.14 mm below figure 7, *B*, in lower part of second node, showing nodal vascular plate, top of arch in scutellum trace, and indications that separation of the second pair of lateral seminal roots from the central stele occurred above this level. *np*, Nodal plate; *sc*, scutellum trace; other designations as in figure 5, *B*. × 44. *B*, 0.144 mm below figure 8, *A*, in the first internode, showing central pith, presence of scutellum trace in the stele, complete separation of second pair of lateral seminal roots from stele, and first indication of vascular connections of first pair of lateral seminal roots. Designations as in figure 5, *B*. × 44.

radicle, plumule, and epiblast are plain. This section bisects longitudinally certain vascular-bundle primordia, the long narrow procambial cells of which (fig. 1, *l*) can be differentiated from surrounding cortical and pith parenchyma. The procambium, being meristematic, takes and retains the safranin stain more than parenchyma, a fact

which also makes it easy to trace the vascular primordia in a well-stained section.

In the central procambial system, between the root and stem portions of the embryo, a section of the procambium extends transversely across the axis (fig. 1, *l*). That this procambial tissue

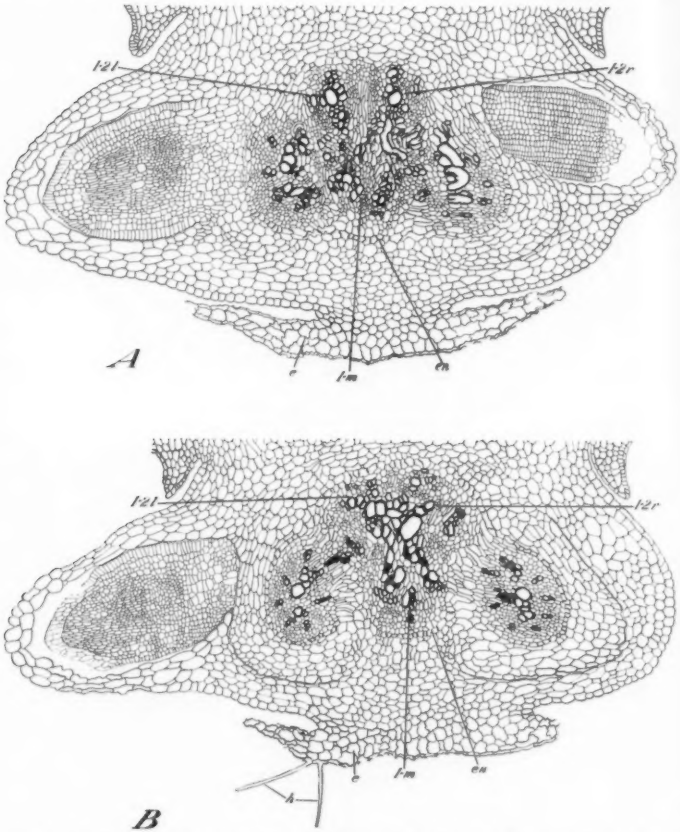


FIGURE 9.—Cross section of the axis (same plant as fig. 5, *B*): *A*, 0.20 mm below figure 8, *B*, in first node, showing epiblast, complete separation from stele of first pair of lateral seminal roots, and origins of bundles *1-m*, *1-2l*, and *1-2r* in first node. *c*, Epiblast; *en*, endodermis; other designations as in figures 5, *B*. × 44. *B*, 0.144 mm below figure 9, *A*, showing connections of bundles *1-m*, *1-2l*, and *1-2r* with primary root, and hair on epiblast. *c*, Epiblast; *en*, endodermis; *h*, hairs; other designations as in figure 5, *B*. × 44.

separates root and stem is evident. This structure later is interpreted as the first node of the young plant.

Extending upward from the cross-axis procambium on the anterior side of the central cylinder is a prominent procambial strand that centers in the well-developed primordium of the first foliage leaf of the plumule (fig. 1, *d*). Similarly, extending upward from the cross-axis structure in the posterior part of the central axis is a procambial strand which, after remaining in the central cylinder for a short

space, diverges outward and into the upper portion of the scutellum (fig. 1, *b*).

Slightly above the point at which the scutellum strand diverges from the central cylinder, a small, narrow procambial strand (fig. 1, *j*) extends part way across the axis from front to back. It connects with the back part of the central cylinder but not with the front. A procambial strand extends upward from this cross strand in the back portion of the central cylinder, ultimately passing into the primodium of the second foliage leaf of the plumule. This second cross-axis procambial structure is hereafter interpreted as the second node of the young plant.

The cross-axis structure just above the divergence of the scutellum trace has not heretofore been thought of as a nodal plate. Lermer and Holzner (*31*) show it in their drawing of the barley embryo but say nothing about it. Sargant and Arber (*50*), in their description of the wheat seedling, speak of the root plate in this region but do not suggest that it marks the position of a node.

Between *l* and *j* of figure 1 is a section of the axis bounded by procambial elements in front and back and with what appears to be a central pith parenchyma (fig. 1, *k*). The procambial strand which leads to the scutellum bounds this region on its posterior side, diverging from the axis just under the second cross-axis plate.

A procambial strand leading from the back of the first cross-axis plate downward into the radicle can be identified. In a stained section the procambium is as easily identified in the root as in the stem axis. Identification is not easy without the stain, however, since the shape of the cells of the root procambium is not sharply different from that of the surrounding tissues. There are commonly an odd number (seven or more of each) of alternating xylem and phloem strands in the young root, the xylem being the more prominent. A median sagittal section of an embryo, such as the one represented in figure 1, bisects

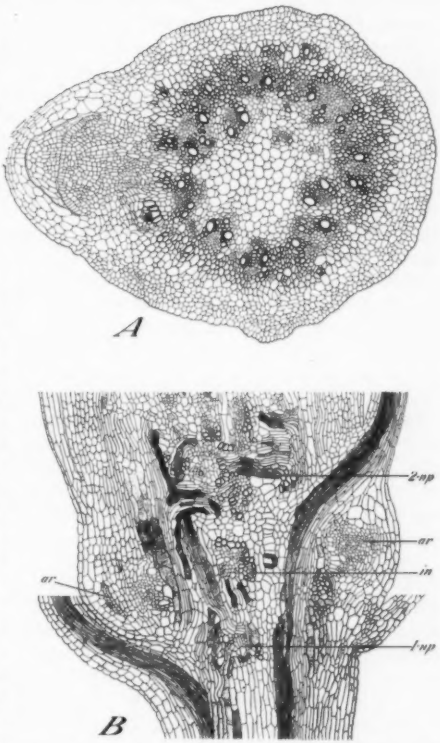


FIGURE 10.—*A*, Cross section of the subcrown internode of a 14-day-old Turkey wheat plant just under the crown node, showing an adventitious root. × 44. *B*, A longitudinal section through the first two nodes of the crown in a 14-day-old Hard Federation wheat plant, showing *1-np* and *2-np*, vascular anastomosing nodal plates, leaf-trace divergences, *ar*, root primordia, and *in*, internodes. × 32.

but one procambial element in the root, which will mature into xylem (posterior side, fig. 1, *l*). The opposite procambial element, which will mature into phloem, is less clearly marked by the form of its cells.

An approximately median longitudinal section through an embryo parallel to its face is shown in figure 2. As is evident from an inspection of figure 1, the orientation of the root and plumule makes it impossible to cut both medianly in the same face section. The section shown in figure 2 is essentially median for the axis proper, although not so for the plumule and not quite so for the primary root. The part of the embryo axis here shown is between the vascular bundles, and the procambium, which will develop into cross-connecting vascular tissue, is not characterized by long, slender cells as is the procambium leading up front and back from *l* in figure 1. It is evidently different, however, from the surrounding pith and cortex parenchyma, and, particularly in a stained section, it can be identified very readily. The two procambial cross-axis structures (fig. 2, *f*, *c*) corresponding respectively to those shown in figure 1, *l*, *j*, are not clear-cut in this view, but they can be identified. The lateral seminal roots, only one of the second pair being present (fig. 2, *d*), plainly originate at or above the procambial plate to which each root belongs.

The lower stem axis region of the embryo changes very little in germination except as its procambium and parenchyma mature into the different tissues of the older plant. Essential features and relationships for this lower region can be fairly well established, even from the limited survey already presented. However, the structure above the second cross-axis plate can be more easily traced after germination and after the development of vascular and other tissues not even suggested in the procambium, etc., of the embryo. This is true especially if there is more than the usual amount of elongation between the second cross-axis plate and the divergence of the coleoptile.

Approximately median sagittal sections through the lower axis region of two 14-day-old plants from just above the divergence of the coleoptile down to and including the transition from root to stem are shown in figure 3, *A* and *B*. The usual amount of elongation in this region is shown in *A*, while much more than the usual amount is shown in *B*. Neither is exactly median, since, even with extreme care it is very difficult so to place a seed that this portion of the axis in the young plant growing from it will be erect and straight. The essential items, however, are shown in both illustrations.

The contrast in elongation between the two plants represented by figure 3, *A* and *B*, shows what may be brought out by growing material under different conditions. In the specimen illustrated in figure 3, *B*, the distance from the under side of the second cross-axis structure (fig. 3, *B*, *k*) to the attachment of the coleoptile at the front of the axis (fig. 3, *B*, *g*) is 1.26 mm. In the specimen on which figure 3, *A*, is based, chosen as representative of the usual response but actually somewhat greater than usual, the corresponding distance (fig. 3, *A*, *k*–*g*) is 0.6 mm. The added length emphasizes vascular structures in this region, although, once recognized, they are perfectly obvious also in the less elongated specimens.

The transition from root to stem (fig. 3, *A*, *n*, and *B*, *n*) is clearly defined. In the embryo it was not easy to place the epiblast definitely

with reference to other structures, but in both of these specimens it is clear that the epiblast, *m*, is so attached to the axis as to subtend the cross-axis structure between root and stem proper (fig. 3, *A*, *n*, and *B*, *n*). The divergence of the scutellum bundle from the central axis, as in the embryo, occurs not at the first but at the second cross-axis structure, divergence beginning just under this second plate, *k*. This is clearly shown in figure 3, *B*. The root diverging from the face of the axis (fig. 3, *B*) has its vascular connections at and above this second plate also. The region between the two cross-axis structures (fig. 3, *A*, *n*, *k*, and *B*, *n*, *k*), with its central pith parenchyma, stands out very clearly. The absence of direct connection between the second plate (fig. 3, *A*, *k*, and *B*, *k*) and the front of the vascular cylinder in the median longitudinal plane is more evident than in the embryo.

Above the second cross-axis structure (fig. 3, *B*, *k*), following an intervening space, is a third anastomosing vascular region (fig. 3, *B*, *i*). While that portion of the axis between the second plate and this structure might be interpreted as an elongation of the second plate, such a conclusion seems hardly logical. Cross-axis anastomosing structures are characteristic of nodes, and there are no known cases of elongation in what may be interpreted as nodes in other regions of the plant in any member of the grass family. Considering that, even with the elongation shown in figure 3, *B*, the axis is still much compressed in this region, the separation of these structures is as definite as in the nodes and internodes of the crown (fig. 10, *B*), which latter no one would question. With the evidence of figure 3, *B*, as a guide, the similarities in figure 3, *A*, are not difficult to trace.

It is interesting to note that, in the specimen from which figure 3, *B*, was drawn, the separation of the coleoptile from the axis was 0.92 mm higher on the scutellum side (fig. 3, *B*, *c*) than on the front (fig. 3, *B*, *g*). The presence of the bud in the axil of the coleoptile on the scutellum side of the axis is plain (fig. 3, *B*, *a*). A root primordium on the opposite side of the axis above the coleoptile divergence also can be seen (fig. 3, *B*, *f*).

A description of the vascular anatomy of that part of a 14-day-old wheat seedling from the vascular plate that separates primary root and stem to the separation of the coleoptile from the axis, as shown in serial cross sections, follows.

In the lowest portion of the seedling axis individual bundles are not differentiated, and without some previous hint even the origins of bundles that are easily identified higher up in the axis can be traced only with difficulty. By proceeding downward from the upper sections, vascular elements can be traced and identified more easily.

A transverse section of the plant at a level slightly below that at which the coleoptile is completely free from the axis is shown in figure 5, *B*. At this level there is no connection between axis and scutellum. In the axis there is a central vascular cylinder of 10 definite bundles with an enclosed central pith. The bundles are all collateral with internal xylem and external phloem. The structure is that generally considered typically internodal.

In the central cylinder opposite the scutellum one bundle is larger and more prominent than the others (fig. 5, *B*, *1–m*). When this bundle is traced upward it is identified as the median bundle of the first foliage leaf. On the opposite side and slightly within the circle

of the outermost bundle ring is a bundle (fig. 5, *B*, *2–m*) that, traced upward, proves to be the medium bundle of the second foliage leaf. A line bisecting these two bundles divides the axis into two equal parts and fixes the plane of axial polarity, the plane with reference to which the leaves and branches of the main axis are alternately and oppositely placed. The longitudinal procambial origins of these bundles are shown in figure 1, and their longitudinal sections are shown in figure 3, *A* and *B*.

In addition to the median bundles of the first two foliage leaves the vascular cylinder contains six additional bundles in the outer ring (fig. 5, *B*, *1–1l*, *1–2l*, *1–3l*, *1–1r*, *1–2r*, and *1–3r*), three on either side of bundle *1–m*. When these bundles are traced upward they prove to be lateral bundles of the first foliage leaf. Slightly within the outer bundle ring, just as is bundle *2–m*, are two other bundles, *2–1l* and *2–1r*, lying between bundles *1–1r* and *1–2r* and bundles *1–1l* and *1–2l*, respectively. These are the main lateral traces of the second foliage leaf, the other laterals of which are derived by the branching of these and other bundles.

On the same side of the axis as bundle *2–m* and just outside the central bundle ring is a group of bundles which, at this stage of development, are smaller than the central bundles and are somewhat indistinctly differentiated (fig. 5, *B*, *cbt*). These are the vascular connections of the bud which develops in the axil of the coleoptile and is attached to the axis slightly above the level of this section.

In sections from this same region in slightly older seedlings adventitious-root primordia are evident (fig. 5, *A*). These are the roots that have been interpreted as a third pair of lateral seminal roots (*43*) but are not represented by primordia in the embryo (fig. 2). Figure 5, *A* and *B*, shows that these roots develop only some time after germination. However, the fact that these roots develop so definitely in this position is important in identifying this general section of the axis as a node, as will be discussed more fully later.

The two coleoptile bundles (fig. 5, *B*, *cl* and *cr*), one on either side, are situated slightly toward the posterior or scutellar side of the axis. Their upward extension is without particular significance in this consideration other than that they are confined to that part of the coleoptile posterior to the vent (pl. 1, *B*). The possible significance of this position of the bundles will be brought out later.

The coleoptile is almost free from the central axis at the level of this section (fig. 5, *B*). It is still slightly joined, however, at the posterior or scutellar side. In the seedling, sections of which are shown in figures 5, *B*, to 9, *B*, the coleoptile was attached to the axis 0.17 mm higher on the back than on the front (on the basis of the number of intervening sections 12μ thick). Attachment at the sides was lower than at either the front or the back (fig. 6, *A*), that on the left side, facing from the scutellum, being 0.36 mm lower, and that on the right side 0.56 mm lower than at the back. In five embryos the posterior attachment of the coleoptile to the axis averaged 0.18 mm higher than the anterior attachment. In five seedlings the posterior attachment averaged 0.49 mm higher. In the seedling, a section of which is shown in figure 3, *B*, as previously noted, the posterior connection was 0.92 mm higher than the front connection. These differences, though not universal (fig. 3, *A*), seem to be typical.

In the specimen under observation, at about the level of the front attachment of the coleoptile, the first indication of a backward divergence in the coleoptile bundles is apparent. This divergence continues, and at the level of figure 6, *A* (0.42 mm lower than that of figure 5, *B*, and 0.26 mm lower than the front attachment of the coleoptile), the coleoptile bundles are well toward the posterior side of the axis. At this level the coleoptile is not yet completely attached to the axis, there being a slight gap on the right side (facing from the scutellum) toward the front. The bundles are still distinct and readily identified, but there is a decided tendency toward an amphivasal condition. There is also anastomosing vascular tissue across the central axis with connection between bundles *1–3l* and *1–3r*, and *2–1l* and *2–1r*. The traces of the axillary bud no longer can be distinguished, and the bundles from which these traces originate, *1–3r*, *2–m*, and *1–3l*, are definitely amphivasal at this level. Chrysler (*8*) notes that in wheat there is an amphivasal condition of the bundles just under and at the nodal plate where branching of the bundles occurs. The structure is suggestively nodelike. This region is shown in longitudinal section in figure 3, *B*, *i*.

At the level of figure 6, *A*, bundles *2–m* and *1–3r* are clearly separate and distinct from each other. At a level 0.10 mm lower, these two bundles, while they still can be identified, show evidence of union. At the level of figure 6, *B*, 0.16 mm lower than that of figure 6, *A*, union is still more distinct. At this same level bundles *1–1r* and *2–1l*, and *1–1l* and *2–1r*, also show evidence of union, or at least of very close association.

At the level of figure 6, *B*, the coleoptile bundles have diverged entirely around and toward the back center of the central vascular cylinder. The first indication of a connection between the coleoptile bundles and the central vascular cylinder is seen in a phloem strand between bundles *cl* and *1–2r*. The development of vascular tissues across the front face outside the central bundles also is evident. The latter is the first indication of the presence of the upper pair of lateral roots, which are connected to the axis just below this region. The general structure suggests an internode rather than a node, varying from the entirely free, open, central pith of the internodes of the culm to about the same degree as do the short compressed internodes of the crown region (fig. 10, *B*). The individual bundles of higher levels can be traced, but there is a strong tendency toward less distinct differentiation.

Between the levels of figures 6, *B*, and 7, *A*, the latter being 0.16 mm lower than the former, bundles (*2–m* and *1–3r*) and *1–3l* have joined. At the level of figure 7, *A*, the three bundles make up one amphivasal complex, in which, however, the phloem elements of the three bundles still can be more or less satisfactorily identified. Bundles *1–1l* and *2–1r*, and *1–1r* and *2–1l*, can no longer be identified as such. Their relative positions in the vascular cylinder are occupied by complexes in which there is no differentiation of individual bundles. Bundle *1–m* is still distinct, and the relative positions of bundles *1–2l* and *1–2r* are still identifiable, largely by reason of xylem aggregates. The coleoptile bundles *cr* and *cl* have diverged still farther toward the back center and their connections, *cr* with bundle *1–2l* and *cl* with bundle *1–2r*, are distinct. The upper, outer part of the upper pair of lateral roots also is discernible in this section, most distinctly

on the left (facing from the scutellum) side of the axis. The heavy vascular system connecting the lateral roots with bundle *1–m* and with the complexes from which originate bundles *1–1r*, *2–1l*, *1–1l*, and *2–1r* is very prominent.

The vascular structure at a level 0.1 mm lower than that shown in figure 7, *A*, is portrayed in figure 7, *B*. At this level the structure is becoming distinctly nodelike. The only bundle that can be identified is *1–m*. Vascular connections extend entirely across the central cylinder, except for a small pith area back of bundle *1–m*. The junction of the two coleoptile bundles, *cr* and *cl*, with the scutellum trace, *st*, occurs at this level, which is also at the top of the arch where the scutellum trace turns backward and downward before entering the scutellum (fig. 3, *B*). The connection between the scutellum trace, coleoptile bundles, and axis is very strongly developed. The roots of the upper lateral pair are now more evident. The heavy tangential xylem elements connecting these roots with bundle *1–m* and other parts of the central vascular cylinder are very prominent across the front face of the stele. This figure represents the transverse structure in the upper part of the second cross-axis vascular plate (fig. 3, *B*, *k*).

The vascular structure at a level 0.14 mm lower than in figure 7, *B*, is shown in figure 8, *A*, this being the lower part of the region shown in figure 3, *B*, *k*. The vascular plate extends entirely across the central cylinder, except for a continuation of the pith area back of bundle *1–m*, noted in figure 7, *B*, and a similar pith area on the opposite side of the axis between the vascular plate and the scutellum trace. That the upper pair of lateral roots are attached above this region is evident from the intrusion of cortical parenchyma between the central vascular cylinder and the root tissue proper (fig. 8, *A*). The section is about on a level with the under side of the arch of the scutellum trace, and the trace is now seen in two parts, the one next to the axis cylinder leading in toward the latter and the other leading down and back toward the scutellum. The scutellum and the axis are joined at this level, complete junction having occurred 0.06 mm higher up.

A cross section of the axis 0.144 mm below figure 8, *A*, is shown in figure 8, *B*. This region is definitely internodal. There is a complete vascular ring with an enclosed central pith. The scutellum trace is now a part of the vascular cylinder. In the vascular cylinder, bundle *1–m* still can be identified. The first indication of the lower or first pair of lateral roots is seen in the heavy vascular development in front of bundle *1–m*. The roots of the second lateral pair are completely separated from the vascular cylinder by a considerable band of cortical parenchyma, showing their lack of vascular connection with this region. The backward and downward extension of the scutellum trace ends at this level. The heavy prominent xylem elements on either side of the scutellum trace in the vascular cylinder are the lower sections of strands that extend upward and in part connect with the coleoptile bundles at their junction with the axis cylinder and in part supply bundles *1–2l* and *1–2r*.

At a level 0.12 mm below that of figure 8, *B*, occurs the first junction of the epiblast with the axis. The tongue or blade of the epiblast extends 0.54 mm above the attachment of the organ to the axis, or to a level slightly above that of figure 7, *A*. This is well above the second cross-axis plate and above the vascular attachment of the second pair of lateral roots. The evidence from this section shows definitely

that the attachment of the epiblast to the axis is at the level of the cross-axis vascular plate between the root and stem, as is also shown in the longitudinal sections (fig. 3, *A*, *B*). In the central cylinder at this point there is a complete vascular plate with no central pith such as shown in figure 8, *B*. The structure at a slightly lower level in the middle region of the cross-axis vascular plate is shown in figure 9, *A*. It is interesting to note that the origin of bundle *1–m* can be identified in this vascular complex. It also is possible to identify the xylem elements, which, higher up, in part supply bundles *1–2l* and *1–2r*, and, in part, supply the axis-cylinder connections of the coleoptile bundles *cl* and *cr*. The stelar structure of the lower pair of lateral roots is evident at this level, plainly showing that their vascular attachments to the axis cylinder are above this cross-axis plate region.

The vascular structure at the very bottom level of the first cross-axis plate is shown in figure 9, *B*. This is 0.144 mm lower than the level of figure 9, *A*. A study of intervening sections shows that bundle *1–m* and the xylem elements of bundles *1–2l* and *1–2r* connect directly with the primary root at the points indicated in figure 9, *B*. The transition from root to stem structure can be seen at this level. In this region occurs the principal transition, since below the protoxylem is exarch, while above, at least in the principal bundles, it is endarch. The protoxylem of bundle *1–m* and of the scutellum trace is endarch immediately above this level (figs. 3, *A*, *B*, and 8, *B*). The radial arrangement of phloem and xylem characteristic of the root is not evident above this point, bundle *1–m* being definitely amphivasal. The structure above this plate is stem, while below it is root.

Nishimura (*41*) noted that the epiblasts of *Poa*, *Phleum*, and *Setaria* bear hairs, which he called root hairs. Similar hairlike structures occur on the epiblast of *Triticum vulgare*, as shown in figure 9, *B*. The writer has observed them also on *T. dicoccum*.

This completes the survey of anatomy in the regions of embryo and seedling essential for this study. As a means of identifying observed structures, a very brief survey of the crown region follows.

An approximately median longitudinal section through the first two nodes of the crown of a 14-day-old seedling is shown in figure 10, *B*. One of the striking features of this region is the anastomosing vascular structure or plate, which is such a prominent part of each node, *1–np* and *2–np*. The not-too-definite vascular arrangement in the greatly shortened internode, *in*, also is clear. The leaf bundles diverging at each node take origin not from the vascular complex of the node, which the leaf subtends, but arise at some node of the axis below, and divergence begins below the nodal anastomosing plate. The edges of two root primordia at the first node, *ar* and *ar*, are above both the leaf attachment and the nodal plate. These roots are not shown in median section, since their location with reference to leaf traces was such that leaf trace and median root view did not coincide. However, enough of the root primordium is present to make it clear that the root attachment is above the nodal plate.

The similarity in outward appearance of the young prophyll and the young coleoptile has already been pointed out. The prophyll has been described many times, and the literature dealing with this structure is extensive. Various views on the prophyll are well summarized by Guillaud (*18*). Percival (*43*) also has pointed out the probable

homology between the coleoptile and the prophyll. In this paper it is not the intention to enter into any extended discussion of the prophyll or its interpretation, but merely to discuss such features as may be apropos to this study.

In cross section (fig. 11, *A*) the prophyll appears as a closed sheathing structure, completely enclosing the young bud. It has a charac-

FIGURE 11.—Camera-lucida outline drawings of cross sections at various levels (*A–H*) through the crown of a 22-day-old wheat plant, showing an axillary bud with enclosing prophyll, prophyll bundles, and origin of the two main prophyll bundles from the bundles of the subtending leaf, the first foliage leaf of the plant. The prophyll bundles of a given shade originate from the leaf bundles of the same shade: *pl* and *pr*, Prophyll bundles; other designations same as in figure 5, *B*. × 26.

teristic two-keeled form with a more or less flattened side in contact with the parent axis, and a curved side in contact with the enclosing leaf. As Bugnon (*5*) has pointed out, this form is an effect of pressure on the young plastic meristematic tissue incident to its position closely pressed between the leaf and axis.

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Figure 11 of the same article, on page 3

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In each keel of the prophyll there is a prominent vascular bundle (fig. 11, *A*). Between these two bundles on the side of the prophyll next to the parent axis there are no other bundles. On the opposite side in that part next the subtending leaf are a number of small secondary bundles which differ in their origin from the two main bundles, connecting directly with what may be termed the first node of the young secondary axis. The ultimate origins of the two principal bundles of the prophyll from the median (*1–m*) and one of the immediately adjacent lateral bundles (*1–1r*) of the subtending leaf are traced in figure 11. The deflection of the axillary bud from the immediately median position can be seen in the position of the prophyll bundles with reference to bundle *1–m* (fig. 11, *E*). Percival (*43*) has fully discussed this deflection.

The coleoptile of *Triticum vulgare* has only two bundles, as shown in figure 5, *B*. Percival (*44*) has noted, however, that the coleoptiles of certain Indo-Abyssinian emmers (*T. dicoccum*) may have from 2 to 6 bundles. Jakovlev and Nikolaenko (*26*) also record that all endemic Abyssinian forms of *T. dicoccum*, *T. durum*, *T. turgidum*, and *T. polonicum* that they examined showed the additional bundles. A diagrammatic cross section of the axis of an emmer with four bundles in the coleoptile is shown in figure 12, *A*. The course of the 2 principal bundles toward the back of the axis and their ultimate junction with the scutellum trace, and that of the other 2 bundles directly into the front of the axis, are shown in figure 12, *B*. The general similarity between this general arrangement and that in the prophyll of *T. vulgare* needs no comment.

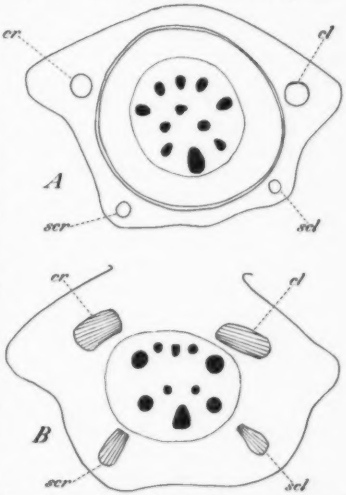

FIGURE 12.—Camera-lucida outline drawings of cross sections of the axis of a seedling of emmer (*Triticum dicoccum*), showing the usual 2 coleoptile bundles of the Graminеae and 2 supplementary coleoptile bundles (*A*) and the divergence of the primary bundles to the back of the axis and of the supplementary pair directly into the front of the axis (*B*). *cl* and *cr*, Primary coleoptile bundles; *scl* and *scr* supplementary coleoptile bundles. × 26.

Avena sativa, *Zea mays*, and *Triticum vulgare* are representative of Van Tieghem's (*59*) three grass-embryo types, which he differentiated on the basis of their behavior during germination. Morphological evidence, indicating that both *Avena* and *Zea* possess the same general anatomical features that have been demonstrated for *Triticum*, is presented for *Avena* in plate 2, *A*, and for *Zea* in plate 2, *B*. The anatomical studies of *T. vulgare* show the validity of properly interpreted external morphology as a basis for suggesting internal anatomy. Incidentally, the essential features in both *Avena* and *Zea* have been checked by anatomical observations, although this evidence is not presented here, since it is not directly pertinent to the present study.

In all three genera the scutellum diverges from the axis, not at the first cross-axis plate but at the second, and the coleoptile diverges at

the third. Their essential similarity already has been pointed out by Avery (*1*), except that he did not identify a second cross-axis plate as intervening between the first plate and coleoptile divergence. The fundamental difference between the three types will be discussed later.

INTERPRETATION

HOMOLOGIES

The embryo is first of all a young plant. Its parts, unless extreme modification has occurred, should show positional and vascular relationships similar to those of their homologues in the plant that develops from it. Such similarities, proved by accurate observations, should be a valid basis for interpreting the structure of the embryo.

In the Gramineae the axis is made up of nodes and internodes, each with its characteristic vascular structure. The appendages of the axis are leaves, axillary buds developing into branches and adventitious roots. The leaves diverge from the axis at the nodes, the buds occur at the nodes in the leaf axils, and in wheat, as in most members of the family, on the same side of the axis as the median bundle of the enclosing leaf although slightly displaced to the right or left of this bundle (*43*). The adventitious roots normally are formed at the nodes above the leaf divergences. There is some deviation in this respect; but in wheat, location of roots at the nodes is the rule rather than the exception. The importance of this root-origin relationship was emphasized long ago by Duval-Jouve (*13*), who said: "Les racines naissent au-dessus des organes foliares; fait trop méconnu par les dessinateurs et peut-être par les descripteurs." The positional and vascular relations of nodes, leaves, and roots are more definite than many have realized.

Adventitious roots, arising from the axis, are normally associated then with nodes and leaf divergences. The constant development of roots in a given definite position on the axis should, therefore, be a very suggestive piece of evidence in identifying a node or an associated subtending leaf. Percival (*43*) considers such evidence valid.

As pointed out, there is a cross-axis vascular plate, marking the transition between the root and the stem of the embryo or the young seedling. In this plate occurs the principal transition from the exarch protoxylem of the root to the endarch protoxylem of the stem. Associated with this plate (fig. 2 and fig. 3, *A*), as with the nodal plates in the crown (fig. 10, *B*), are a pair of roots and the subtending scale-like epiblast. It is interpreted, therefore, as the first node.

The epiblast is entirely devoid of vascular tissue, a fact that has led some (*1*, *50*) to conclude that it is a mere outgrowth of the coleorhiza or axis and of no morphological significance. Others (*7*, *65*) have considered it homologous with the auricles of the seed leaf. Its position, subtending as it does the first node with an internode intervening between it and the divergence of the scutellum, seems to rule out the latter contention. Still others (*4*, *43*, *45*, *60*, *63*) have considered the epiblast a rudimentary leaf.

It has been shown (fig. 10, *B*) that in wheat the vascular bundles of a leaf subtending any given node do not originate in that node, but rather in a lower node. This fact has been repeatedly illustrated in other plants, both monocotyledons and dicotyledons (*15*, *55*). In all cases leaf traces arise in a stem node and in no case from a root.

Position at the first node, with only root tissue below the node, precludes vascular leaf connections in a leaf subtending this node. The epiblast resembles a leaf in its divergence from the axis at a node, in the association of adventitious roots with it and its node, just as with any other leaf and node, and in its position distichous with the next succeeding leaf. Therefore, the epiblast is interpreted as a vestigial first leaf without vascular tissue, because of its position at the first node.

It might be argued that the hairs noted as occurring on the epiblast (fig. 9, *B*) indicate a root origin for this structure. It seems just as logical to consider them as homologues of the trichomes or leaf hairs.

In the wheat embryo and seedling there is a very definite second node with nodal plate, diverging trace (scutellum trace), and accompanying adventitious roots. All of these structures are plainly shown in figures 1, 2, 3, *A*, *B*, and 8, *A*. The relation is identical with that shown for crown nodes in figure 10, *B*. The scutellum trace diverges from the axis just below the definite vascular plate with which adventitious roots are associated as in any typical node. This is identical with the divergence of other leaf traces in wheat. The scutellum, therefore, is interpreted as a leaf whose vascular connection is reduced to one median bundle divergent from the second node. It is a second leaf that alternates with the first nonfunctional leaf, the epiblast. It is the functional seed leaf, the cotyledon.

It has been shown that roots are regularly formed above the divergence of the coleoptile. A bud also develops in the axil of the coleoptile. A cross-axis vascular structure (fig. 3, *A*, *i*, *B*, *i*) also is present. It is true that in the specimen from which figure 3, *B*, was drawn, this cross-axis structure is well below the separation of the coleoptile from the axis, but it is above the divergence of the coleoptile bundles and their connection with the axis and the scutellum trace. In the more typical specimen (fig. 3, *A*) the separation of the coleoptile from the axis is more nearly the same in relation to this cross plate as that in typical crown nodes (fig. 10, *B*), the principal part of the plate being above the leaf attachment. It is considered that vascular plate, divergence of the coleoptile, roots, and bud identify a node, the third of the young plant. The structure diverging from the axis at this node, the coleoptile, is interpreted as the third leaf of the plant.

In the Gramineae every bud, terminal or lateral, is protected by a closed, sheathing structure. For the terminal bud this structure is the coleoptile, and for each lateral bud a prophyll. The marked resemblance between coleoptile and prophyll cannot be fortuitous. Each occupies the position of an external sheathing leaf, enclosing and protecting a bud. Their forms are similar (pl. 1, *B* and *C*). Each is without blade. Each has two opposite vascular bundles, one on each side, slightly toward the back of the structure. In each these two bundles take their origin from the bundle or bundles of the subtending leaf. While the prophylls possess additional bundles, and the coleoptiles of most of the Gramineae, including *Triticum vulgare*, do not, the additional bundles in the coleoptile of some forms of *T. dicoccum* indicate that this difference is an evolutionary one that does not separate the two fundamentally. Differences in vascular connection can be accounted for by position with reference to the main axis, as also can the position of enclosed axillary buds. Vascular connections of a structure connecting back to an axis on which the

structure is terminal must differ from connections of a similar structure lateral to the axis. The position of buds axillary to the coleoptile or to the prophylls is determined by the polarity of the enclosed axis. The polarity of the coleoptile is that of the main axis, and the bud is between the two bundles of the coleoptile. The polarity of the prophylls is that of the lateral axes, at 90° from that of the parent axes, and the buds are in line with the prophyll bundles.

Because of its similarity to the prophylls in position, form, vascular anatomy, and function, the coleoptile is interpreted as the homologue of a prophyll (*43*). The final interpretation of the relationships of both to other leaves is reserved for later study.

The presence of the bud in the axil of the coleoptile on the same side of the axis as the scutellum raises a consideration that cannot be dismissed lightly. The evidence submitted shows that the scutellum diverges from the second node and the coleoptile from a third, or at least from a node above the second. If the relation of the coleoptile to preceding leaves is the same as with foliage leaves, the alternate distichous phyllotaxy of the Gramineae requires that the bud be on the side of the axis away from the scutellum.

It is possible that the coleoptile does not bear the same phyllotaxic relation to preceding leaves as prevails for the foliage leaves. As the first leaf enclosing the terminal bud, it may be independent of the scutellum. If this is true the position of the bud simply indicates the starting point, so far as the axis enclosed by the coleoptile is concerned.

Certain observations suggest another possible explanation. It was noted that the coleoptile is attached to the axis higher on the back, or scutellum side, than on the front (figs. 3, *B*, and 5, *B*). It was also noted that in some cases connections on the right and left sides were lower than on either back or front. The position of the coleoptile bundles toward the scutellum side of the axis also was noted, as well as the position of the vent on the front face of the scutellum and in front of these bundles.

Hanstein (*20*) observed in *Brachypodium* that in the development of the coleoptile in the young embryo a projection is first formed between the scutellum and the growing point. Very slightly later a similar projection appears in the form of a half collar on the lower side of the growing point. These two projections stand opposite each other like lips. As they grow they merge and form the sheathing coleoptile. The writer has observed a few young barley embryos that suggest this same sequence of events, although the evidence was not conclusive.

The observed situation in wheat in the attachment of the coleoptile to the axis and in the placement of its vascular bundles, together with the above-noted interpretation by Hanstein, suggests that the coleoptile may represent a structure derived originally from two-leaf primordia, the anterior portion representing a leaf alternate with the scutellum, and the posterior part representing a similar leaf alternate with the other. The placement of the two bundles toward the back of the coleoptile, there being no connection with the front portion, suggests a separate origin for the two parts. The additional bundles of *Triticum dicoccum* might suggest that the main pair and the supplementary bundles each belong to what may originally have been separate primordia. If the coleoptile represents an evolutionary

equivalent of two leaves, the position of the axillary bud on the scutellum side of the axis is in accord with the regular phyllotaxy of the

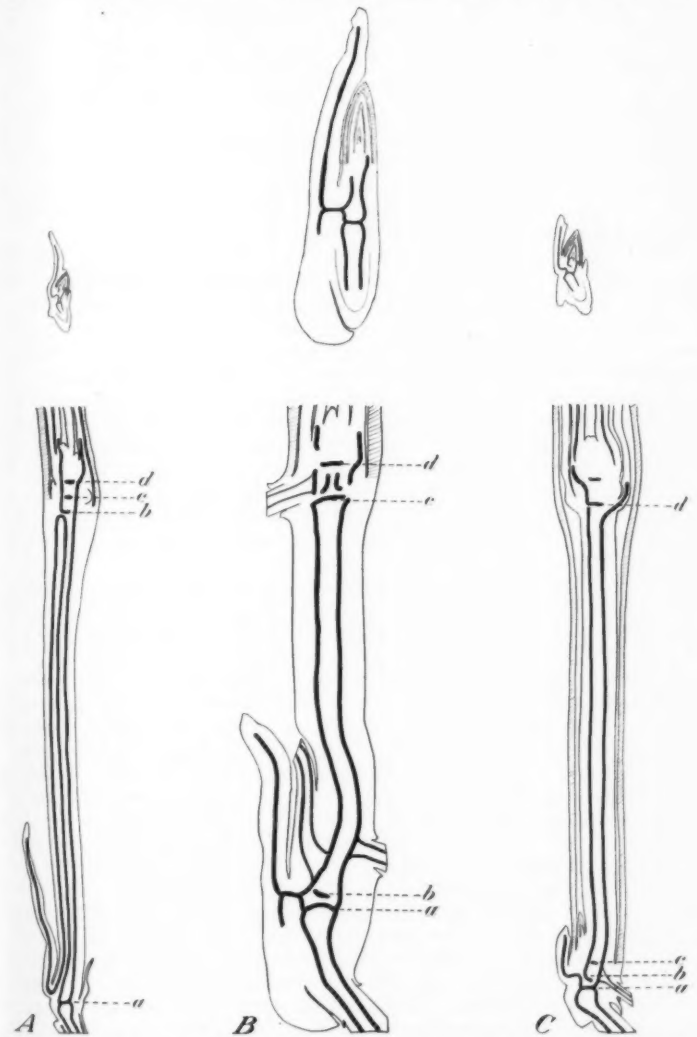

FIGURE 13.—Diagrammatic sagittal view of the embryos (above) and the subcrown region (below) of oat, corn, and wheat seedlings, showing vascular and nodal relationships and the differences in intercalary growth during germination in the three genera. × 4. *A*, Oat (*Avena sativa* var. Iogold): *a*, First node, transition from root to stem; *b*, second node, scutellum-trace divergence; *c*, third node, coleoptile divergence; *d*, fourth node. *B*, Corn (*Zea mays* var. Garrick): Designations the same as in *A*. *C*, Wheat (*Triticum vulgare* var. Turkey): Designations the same as in *A*.

Gramineae. If this hypothesis is correct it would follow that the coleoptile node is likewise the equivalent of two nodes. The general situation in the third node (fig. 3, *A*, *i*, *B*, *i*) might suggest this as a

possibility. The two opposite buds often occurring in the axil of the prophyll also might support such a hypothesis.

The order of development of the parts of the coleoptile, the back first and the front very slightly later, might be urged against the above-mentioned hypothesis. Percival (*43*) gives the order of differentiation in the wheat embryo as follows: (1) Scutellum; (2) coleoptile, as a ring, the upper half more rapidly than the lower; (3) the rudimentary first foliage leaf; and (4) the epiblast. If the epiblast is really positionally a first leaf it must follow that the order of differentiation in the embryo is not important in place determination. Coulter and Chamberlain (*11*) point out that flower parts do not necessarily appear in acropetal succession, but that positionally higher members may appear before positionally lower ones.

The writer does not consider the evidence in support of the dual nature of the coleoptile as more than suggestive. Hanstein's (*20*) evidence as to the origin of the coleoptile from two primordia is not so convincing as to be final, although failure to prove Hanstein correct would not of itself necessarily invalidate the hypothesis here suggested. The structure and the vascular relationships of the coleoptile of wheat are not against the hypothesis, but without additional proof it cannot be considered more than an interesting possibility. The evidence for interpreting the divergence of the scutellum as occurring from the second node and that of the coleoptile from a third node seems conclusive. There must be a reason for the location of the coleoptile bud on the scutellar side of the axis. Any definite conclusion as to this reason, however, must be reserved for further study.

As pointed out previously, the embryos of *Avena sativa*, *Zea mays*, and *Triticum vulgare* are similar in fundamental anatomy. In all three the first node marks the principal transition between the primary root and the stem axis, the scutellum diverges from the second node, and the coleoptile from the third node. The essential differences in the three types lie in the location of intercalary growth during germination and early seedling development. This fact was pointed out by Avery (*1*), but, not recognizing the basic structure, he did not definitely determine the locus of intercalary growth in *Avena* and *Zea*.

Intercalary growth in *Avena* occurs in the first internode of the axis. It involves the entire cross section of the axis, including that part of the scutellum trace in the axis, but it does not involve the scutellum. The result of this growth is a lifting of the second node and with it the scutellum trace, resulting in an elongated first internode and in the divergence of the scutellum trace from the second node at a point high above the attachment of the scutellum to the axis. This is the reason for the inverted scutellum trace extending from the point of divergence parallel to the axis downward to connection with the scutellum. The second internode usually elongates very little, and the second and third nodes, from which the coleoptile diverges, are very close together. The identity of each is externally evident, however, from root origins and coleoptile divergence (pl. 2, *B*). In *Avena* the second and third nodes are often in close proximity to the crown, so close in some cases that the second node might be interpreted as the first node of the crown.

In *Zea* intercalary growth occurs not in the first internode, but in the second. The third node, the node of coleoptile divergence, is

lifted and separated from the second node. Being above the divergence of the scutellum, intercalary growth does not involve the scutellum trace. In *Zea* the third or coleoptile node is in close proximity to the crown, of which it might be considered the first node.

In *Triticum* there is ordinarily little intercalary growth in either the first or second internodes, the third being the first internode in which such growth is important. Consequently, the coleoptile node is not lifted away from its original position in the embryo and the coleoptile remains in close apparent connection with the scutellum. Even when some intercalary growth does occur in the first and second internodes (fig. 3, *B*) it is not enough materially to modify external morphology. Internally, such intercalary growth may produce a limited arching or inversion of the scutellum trace (fig. 3, *B*). In *Triticum* the fourth node, the node of the first foliage leaf, is usually the first node of the crown. The contrasts in the three types are shown diagrammatically in figure 13.

DEVELOPMENTAL ANATOMY AND MORPHOLOGY

The procambial system of the embryo and the vascular system of the seedling that develops from this procambium are well designed to serve the young plant during and after germination. The developmental sequence during germination, as well as certain developmental responses in the seedling, can be understood and interpreted on the basis of this conducting system. A diagrammatic view of the vascular structure in the wheat seedling is shown in figure 14.

During germination the young plant is entirely dependent on food supplies transported from the endosperm of the seed. The scutellum with its absorbing epithelium is provided with a fan-shaped system of vascular strands, ramifying throughout its entire extent (*1*, *43*). These strands connect with the main central scutellum bundle near its upper extremity. The scutellum trace then serves as a direct passageway for food transport into the axis.

The main body of the scutellum trace entering the axis connects downward directly with the first node. There is a well-developed connection between the scutellum trace and the coleoptile bundles. There is direct connection through the coleoptile bundles with bundles *1–2l* and *1–2r* of the central axis just above the second node. Certain strands of this connection also lead into the anastomosing vascular complex which connects with bundles *2–1l* and *2–1r* of the second foliage leaf.

It has already been pointed out that in early germination the coleorhiza and the primary radicle first show evidences of growth, quickly followed by the plumule, but at a somewhat slower rate. Very shortly after the primary root has emerged the first pair of lateral roots appears; somewhat later the second pair appears, and still later the root on the face of the axis appears. This sequence could be explained in part by the relative development of the different parts in the embryo (fig. 2). That this is not the sole reason, however, is shown by the increasing frequency of failure of members to develop at all with each advancing stage in the sequence. Taylor and McCall [8] found that in a majority of cases only three seminal roots developed, the primary and the first pair of laterals. In another fairly large

[8] Taylor, J. W., and McCall, M. A. See footnote 7.

number of cases only four seminal roots developed, the primary, the first pair of laterals, and one of the second pair. In a number of cases five roots developed, the primary, the first pair of laterals, and both of the second pair. As a rule, only relatively few seedlings of most varieties of wheat have the full potential complement of six seminal roots. While the number of seminal roots that develop past the primordial stage varies with seed maturity and quality, environment,

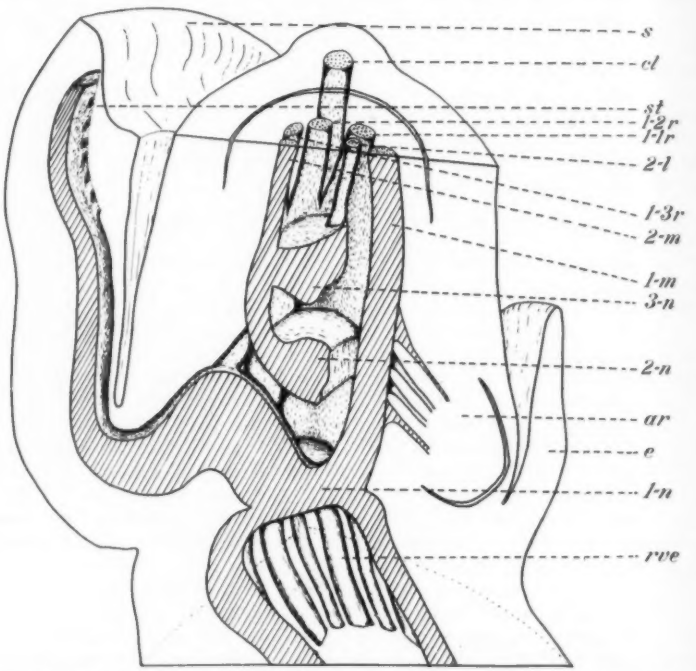

FIGURE 14.—Diagrammatic perspective sagittal view of the vascular elements in the axis of a wheat seedling between the transition from root to stem and the divergence of the coleoptile. × 40. The specimen upon which the sagittal face view of this drawing is based shows somewhat more than the normal elongation in this region of the axis. Xylem and phloem of the conducting tissues are not differentiated. (Nodal plates are shown as solid structures, which is not true for that of the third node.) In the root, the strands beginning with the large strand on the posterior side are alternately xylem and phloem. The branches of the scutellem trace are not shown, except their origins: *s*, Scutellum; *cl*, coleoptile bundle (left); *st*, scutellum trace; *1–3r*, second lateral trace of first foliage leaf; *1–1r*, first lateral trace of first foliage leaf; *2–l*, lateral trace of second foliage leaf; *1–3r*, third lateral trace of first foliage leaf; *2–m*, median trace of second foliage leaf; *1–m*, median trace of first foliage leaf; *3–n*, vascular complex of third node; *2–n*, vascular complex or plate of second node; *ar*, adventitious root; *e*, epiblast; *1–n*, vascular transition from root to stem (first node); *rve*, root vascular elements.

and variety, vascular anatomy bears a very intimate relation to the final result.

As the food supply from the endosperm is conveyed along the scutellum trace, its most direct routes lead to the first node and into the coleoptile. The portion of the scutellum trace passing on to the first node is much larger than the branches leading to the coleoptile. The connection of this large trace to the first node, which is completely connected with the primary root, insures the root being well supplied with material for growth. First growth is evident in the pri-

mary root and in the coleoptile, and, as might be expected, is more rapid in the former. As the food moves into the first node it immediately enters a most efficient cross-axial conducting system, which connects with all bundles of the root axis. The coleoptile, having only two bundles and being dependent on diffusion to a greater degree, naturally grows somewhat more slowly, in spite of its direct connection with the food supply.

It has been pointed out that the first pair of lateral roots are associated with the first nodal plate. In common with the primary root they are in a position to draw directly on the labile food supply conducted by the scutellum trace into the first node. The advantage of this placement is shown by the fact that this pair of roots emerges immediately after the primary root and that they develop in the great majority of cases. The growth of this pair of lateral roots also is almost as rapid as that of the primary root, and all three of these roots ultimately reach much the same degree of development.

The second pair of lateral roots, associated with the second node, is much less favorably situated with reference to food stored in the endosperm. To reach these roots food materials must pass either from the first node, where the requirements of the primary root, the first pair of lateral roots, and, as will be shown later, the first foliage leaf, reduce the total, or through the relatively smaller connections from the coleoptile bundles into the axis. In the latter case the second lateral roots also must compete with such other structures as the coleoptile, the axis itself, the plumule, etc. That the amount of food material available through these channels is not always adequate is shown by the fact that in a large number of cases neither member of the pair develops, while in many others only one grows. When this pair of roots does develop, their slower growth shows the result of their less favorable position.

The direct connection of the median bundle of the first foliage leaf with the first node has been noted. There also is a direct connection across the first node from the scutellum trace to this bundle. It is apparent that any nutritional advantage favoring the rapid development of this first leaf, the first synthesizing organ of the young plant, is advantageous. The vascular structure insures this bundle a favorable connection both with labile food materials from the endosperm and with soil nutrients. The sharp separation of the median bundle from the second node, with such insurance as this may give that no food material shall be diverted from the young leaf at this level, is also undoubtedly significant. In the evolutionary sequence variants possessing this characteristic would seem to have a greater survival potential than others possessing a structure allowing for greater diversion.

The connection of the second leaf and of the growing point with the endosperm food supply through axial connections with the coleoptile bundles is also significant. That these structures are able to divert a considerable part of the food materials conveyed through this channel is indicated by the relatively poor development of the second pair of lateral roots in so many cases.

It is a suggestive fact that the sixth seminal root, located at the second node on the front face of the axis, develops less frequently than the others, and then usually later and less vigorously. Nutritional supplies for this root during the germination period are very

evidently less abundant than those for the others. While this root is directly connected with the median bundle of the first foliage leaf (fig. 3, *B*), its later development suggests that the upward nutritional stream from stored sources is not extensively diverted to this root, but rather that its growth is chiefly dependent on material synthesized later by the first leaf.

Taylor and McCall [9] have shown that in seedlings grown at high temperatures, when growth and use of endosperm food supplies both are rapid, the bud in the axil of the coleoptile fails to develop. At low temperatures, when growth is slow, this bud develops in a large percentage of cases. It is very evident that the development of this bud is dependent on the availability of stored food supplies. As noted in figure 5, *B*, the vascular connections of this bud originate from bundles *2–m*, *1–3l*, and *1–3r*. The connections of the coleoptile bundles with the stele (fig. 8, *A*) make rather slight union with the vascular complex from which these bundles originate. When respiration and growth are relatively slow, as at lower temperatures, the stored food is naturally used more slowly, and there is a transfer and secondary storage of food materials in the cortex of the seedling itself. When this condition exists food materials reach bundles *2–m*, *1–3l*, and *1–3r* in such quantity as to induce the development and growth of vascular connections for the coleoptile bud. With more rapid use of stored food the connection is so inadequate that there is an insufficient supply of food materials reaching these bundles. Consequently, the vascular connections for the coleoptile bud are very poorly developed. This is true to such an extent that, even after the crown leaves have grown to a point at which they are synthesizing excess food materials, these materials cannot be utilized by the coleoptile bud because of inadequate primary vascular connections. The presence or absence of adequate vascular connections is apparently the factor that finally determines the development of this bud.

The subcrown root system of the wheat plant does not ordinarily play so large a part in the later life of the plant as does the crown-root system, nevertheless it does play an important role. As previously noted (*33*), under certain arid conditions crown roots fail to develop. The subcrown system in such cases is capable of carrying the plant through its life cycle to complete maturity. The plant does not tiller to any extent under these circumstances, and in many instances only the main culm develops fully. The main culm, in all but size, is entirely normal, however, except for a tendency to lodge because of the absence of the usual anchoring supplied by the crown roots. The connection of the bundles of the primary axis with the primary and lateral roots is direct, and, despite the restricted extent of these roots and the limited cross section of the subcrown axis, the end result demonstrates that roots and axis together are a highly efficient absorbing and conveying system. The observations of Simmonds and Sallans (*54*) and Todaro (*61*) emphasize the importance and efficiency of this system.

DISCUSSION

A fact advanced as a principal reason for interpreting the coleoptile as an extension of the ligule (*7*), the leaf of which the scutellum is the blade, or of the sheath (*2*) of this leaf, is its direct vascular connection

[9] TAYLOR, J. W., and MCCALL, M. A. See footnote 7.

to the scutellum trace. Several items of evidence fail to support such a view. Percival (*43*) notes that, in wheat, for example, the ligules and auricles are without vascular elements. The proof offered that even in wheat there is an internode intervening between scutellum and coleoptile divergence suffices to discredit such an interpretation. The supplementary bundles of *Triticum dicoccum*, which do not connect with the scutellum trace, completely clinch the case. If these supplementary bundles were really part of a leaf of which the scutellum is another part, they should originate below the second node and should diverge from the axis at the same general level as the scutellum trace. Actually, these bundles diverge above the second node (fig. 12), thus proving conclusively that the coleoptile is not a part of the scutellum. Percival (*44*) also interprets these bundles as proving the separate identity of the coleoptile, but he bases his opinion on other reasons than the divergence of the bundles above the node of scutellum-trace divergence.

Differences in the location of intercalary growth in Van Tieghem's (*59*) three embryo types, which have been pointed out, seem to be strong confirmatory evidence in support of the interpretation of embryo structure and homologies herein presented. In the Gramineae, in all portions of the plant above the embryo, intercalary growth occurs only in the internodes. There is no reason to expect this condition to be materially modified in the embryo. The intercalary growth that occurs in *Avena*, below the divergence of the scutellum trace from the axis, in itself indicates that the portion of the axis below this divergence is internode. Similarly, the intercalary growth normally occurring in *Zea* between scutellum and coleoptile divergence argues that this section likewise is internode and not an elongation of the node of scutellum divergence, as maintained by Worsdell (*65*), Howarth (*25*), and others. The latter reasoning also applies to *Triticum*. If these sections of the axis showing intercalary growth are internodes, then scutellum divergence must be from the second node and coleoptile divergence from the third node.

The theory of Sargant and Arber (*50*) that the mesocotyl represents an evolutionary fusion of the stalk of the scutellum with the hypocotyl of a hypothetical epigeous ancestor does not seem to be in accord with the facts. These authors were evidently confused by the structure in *Avena*, the explanation of which has been given above. So far as vascular connections, etc., are concerned, there are no fundamental differences between *Avena*, *Zea*, and *Triticum*. The essential difference between the three genera is in the location of important intercalary growth in one or another of the three lower internodes. There is certainly nothing in the structural development of *Triticum* or of *Zea* to support the fusion hypothesis, nor is there any necessity for so interpreting that of *Avena*.

A theory recently has been advanced by Boyd (*3*) to the effect that the scutellum of the grasses represents the evolutionary fusion of a sucker, such as that of *Hedychium*, with part of a sheathing cotyledon, the latter, in effect, appressed over the face of the former. The anatomical evidence submitted in the foregoing pages does not require such a complicated theory to explain the case. The vascular connections of the scutellum diverge from the axis at a node, exactly as do those of any other leaf. On the basis of leaf relations in the grasses

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there is no reason to consider the scutellum as anything other than a leaf. It seems unnecessary to develop a theory involving anything more complicated than modification of a leaf to account for the facts.

Boyd (*3*) has called attention to the fact that the term "mesocotyl" is misleading, implying, as it does, location in the "middle" of the cotyledon. In maize the so-called mesocotyl is actually epicotyl, as it is in wheat when it develops. In oats, on the other hand, what is called the mesocotyl is actually hypocotyl. Even though the term be incorrect, some might consider its continuation desirable because of its wide usage. However, no argument can make it seem logical or useful to use an incorrect term synonymously for two entirely different structures. It would seem better to abandon the term entirely and to designate correctly that part of the maize axis heretofore called mesocotyl as epicotyl, and that part in oats called mesocotyl as hypocotyl.

Failure to recognize that the scutellum diverges from the second rather than from the first node has been responsible for much confusion in interpreting the grass embryo. It has been responsible for the opinion that the epiblast is a rudimentary leaf diverging from a second node. It was undoubtedly responsible for the suggestion of so sound an observer as Percival (*43*) that the seminal root on the face of the second node represents a former bud in the axil of the epiblast. Actually, this root is only a third root at the second node, such a root very commonly occurring opposite the median point of the subtending leaf at any node.

In interpreting the grass embryo there has been a tendency to confuse the issue in some degree at least through overemphasis of evolutionary relationships. If all the facts were available, recognized, and properly interpreted, the evolutionary sequence would undoubtedly tell the complete story. Yet, without all the facts, the overstressing of evolution has led some at least to overlook the significance of anatomical features that are present and to think of the grass embryo as much more modified in its structure and parts than is actually the case.

In all questions of interpretation it seems logical to expect that the simplest explanation, in line with known laws, that fits the observed facts is most likely to be correct. Theories that involve structures or development not usual or readily discernible in the family under consideration are very likely to be questionable.

SUMMARY

A study of the crown and upper portions of *Triticum vulgare* shows a definite positional and vascular relationship between nodal vascular plate, leaf and leaf-trace divergence, and root and axillary-bud origins. Leaves diverge from the axis at the nodes. Leaf traces originate from some node in the axis below the node from which divergence occurs, and divergence begins below the nodal plate. Roots originate at the nodes above leaf divergences, and their principal vascular connection is above the nodal plate. Axillary buds originate at the nodes in the leaf axils, also well above the nodal plate. Similar relations prevail in the embryo.

The cross-axis procambial plate separating primary root and stem in the embryo is the first node of the young plant. Associated with

this node are roots and the divergence of the epiblast. Because of its association with a node and with root origins and its alternate distichous position with reference to the next succeeding leaf, the epiblast is interpreted as a vestigial leaf. It is without vascular connection because of its position at the first node, below which is root tissue, in which leaf traces do not originate.

Immediately above the first node is a short internode with enclosing procambial cylinder and central pith parenchyma. Terminating this internode is a cross-axis procambial plate, the second node of the embryo. Immediately under this nodal plate the scutellum trace begins to diverge from the axis. Roots take origin above the nodal plate. Because of its association with nodal plate and root origin, and the divergence of its trace from the axis below the nodal plate, as with any other leaf, the scutellum is interpreted as a leaf, the functional cotyledon, divergent from the second node.

Above the second node is a second internode terminated by a third cross-axis vascular structure. Associated with this structure are the divergence of the coleoptile, and root and axillary-bud origins above the coleoptile divergence. This region is, therefore, interpreted as the third node of the young plant, and the coleoptile as a third leaf. Because of its similarity to the prophylls in function, position with reference to a plumule, outward appearance, and vascular anatomy and connections, the coleoptile also is interpreted as the homologue of the prophyll.

The higher attachment of the coleoptile on that side of the axis toward the scutellum, the vascular relationships of the coleoptile in *Triticum vulgare* and *T. dicoccum*, and Hanstein's observations on the origin of the coleoptile in *Brachypodium* as two opposite projections that later merge into the coleoptile ring, suggest that the coleoptile may represent the evolutionary equivalent of two leaves. In such a case the third node would represent two nodes. If correct, this explains the position of the bud in the axil of the coleoptile on the same side of the axis as the scutellum, when the phyllotaxy of the Gramineae would seem to require it to be on the opposite side. The evidence in support of this hypothesis is considered only suggestive.

The structure of Van Tieghem's three types of grass embryos as exemplified by *Avena*, *Zea*, and *Triticum* are interpreted as being fundamentally similar. In all three the scutellum diverges from the second node and the coleoptile from the third node. The difference between the three lies in the internode location of important intercalary growth during seedling development. In *Avena* this important growth occurs in the first internode, in *Zea* in the second internode, and in *Triticum* in the third internode. The location of intercalary growth in these three embryo types is considered in itself as identifying the internodes and confirming the interpretation of the embryo herein presented.

Embryo and seedling anatomy in the Gramineae does not justify the term "mesocotyl." The structure called mesocotyl in *Avena* is hypocotyl, while that called mesocotyl in *Zea* is epicotyl. The term "mesocotyl" should be abandoned and the correct terms used instead.

Seedling morphology and developmental sequence are determined in large part by vascular anatomy. The primary root, the coleoptile and plumule, and the first foliage leaf are most favorably situated from a vascular standpoint to use food material stored in the endo-

sperm. The first pair of lateral seminal roots also is favorably situated, while the second lateral pair of seminal roots, the face seminal root, and the coleoptile axillary bud are each successively less well situated to use food materials stored in the endosperm.

The primary root system, because of its direct connection with the primary axis, is most effective in absorbing soil nutrients and conducting them to the crown of the plant. Unless destroyed by disease or in some other way, the roots of this system, the seminal roots, remain functional during the life of the plant.

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SELF-FERTILIZATION IN SUGAR BEETS AS INFLUENCED BY TYPE OF ISOLATOR AND OTHER FACTORS[1]

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INTRODUCTION

Self-fertilization in sugar beets (*Beta vulgaris* L.) has been commonly obtained in breeding work by "space isolation" of the plants in gardens and fields. Work of this type has been carried on by investigators in the Division of Sugar Plant Investigations, and lines of sugar beets with records of having been inbred for eight generations are now available. As a means of avoiding off-pollination, it has been the practice since 1929 to have a space of at least one city block separating all plantings.

Certain difficulties are inherent in the space-isolation method of selfing sugar beets. Unless each location is visited frequently for purposes of cultivation, irrigation, and general care of the isolated mother beets, the losses become considerable. Such frequent visits are costly, however, and even with the best of care there is always some loss due to depredation by poultry, dogs, and other trespassers. Unless the irrigation is favorable, the viability of the seed produced may be affected, as shown by Overpeck and Elcock (*9*),[3] who found a direct and positive correlation between irrigations at 1-, 2-, and 3-week intervals and the percentage of germination. Perhaps the most important drawback inherent in space isolation is the danger from cross-fertilization with chard and red beets which have overwintered in gardens with sugar beets in commercial seed fields, or with isolated plantings of sugar beets retained for breeding purposes.

In addition to these difficulties, in using the isolation method one is limited by the lack of suitable locations available. In the work conducted by the writer's colleagues, at Fort Collins, Colo., and vicinity, it was necessary to use 400 to 500 locations as far as 15 miles from the station in order to obtain isolated plantings.

In order to facilitate breeding experiments by the procedure that involves selection in self-fertilized lines, it is exceedingly important to develop some method other than isolation by space, whereby selfed seed in sufficient quantities for breeding work may be produced. The studies reported herein were planned to determine some of the physiological factors affecting seed setting and the comparative value of different types of bags or cages as isolation agents.

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[2] The writer wishes to express his appreciation of the suggestions and criticisms given by A. W. Skuderna, principal agronomist, and G. H. Coons, principal pathologist, Division of Sugar Plant Investigations, during the progress of this work and in connection with the preparation of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 337.

REVIEW OF LITERATURE

Shaw (*11*) found that the beet flower is self-sterile if its pollen is saved and pollination made later when the stigma is receptive, but that there is no sterility between flowers on the same plant. The flower has been considered protandrous and for that reason the individual flower is ordinarily not selfed. Artschwager (*3*) has found, however, that, in rare cases, the shedding of the pollen and the opening of the stigmatic lobes may occur simultaneously. Wind and insects are considered to be responsible for the transfer of pollen between flowers.

Shaw (*11*) and Artschwager (*3*) consider thrips to be the most active pollenizers.

Reed (*10*) obtained a very small amount of seed from beets which flowered in isolation, but when the plants were covered loosely with a bag which allowed insects to enter, good seed setting was obtained.

Nilsson (*8*) worked out a relatively easy and effective method for selfing sugar beets and mangels. A parchment bag about 5 by 15 inches in size was used. In order to obtain more even ripening, the ends of single branches were cut off. Any lower flowers that were already open were removed and a single branch was then enclosed in each bag. A piece of cotton was wrapped around the stem and the bag tied around the cotton; the upper end of the bag was held out by a little stick fastened to the corners; and both the stick and the lower end of the bag were then tied to a bamboo stake. Good results were obtained when this method was used. The first isolations were made in 1916. Of 168 plants selfed in that year, each of which yielded sufficient seed to obtain a progeny test, 47 to 49 percent produced what appeared to be homozygous progeny.

Vilmorin (*13*) reported the production of sugar-beet seed on mother beets isolated within cloth cages. The cages were shaken each day to assist pollination. Archimovitch (*2*) used cotton-cloth cages with glass windows to cover entire plants in isolation in comparison with vegetable-parchment wrappings on single branches, and obtained better results with the cages. The studies were reported for a 5-year period, and essentially the same results were obtained for each of the last 4 years when comparisons were available. Some evidence was obtained which indicated that the ability of a plant or of individual isolated branches to set seed when isolated under a cage was hereditary in nature.

Stewart and Tingey (*12*) reported results obtained in 1926 from experiments in which they used from 5 to 10 grocery bags of 2-pound size on each of about 75 plants. In some cases single branches were enclosed in a single bag and in other cases several branches were so enclosed. Cotton was not wrapped about the stems in these tests. Striking differences were observed between plants, with respect to their ability to set seed.

Down and Lavis (*5*) reported that the space-isolation method was effective for practical breeding at East Lansing, Mich., and their tests in which vegetable-parchment bags were used on about 30 plants resulted in a total failure to obtain seed. They concluded that this failure may have been due to the very high temperatures that prevailed during the pollinating season.

METHODS AND EQUIPMENT

For the most part, mother beets selected from commercial varieties were used for these studies. A few roots were used that had some inbreeding.

The bags employed for isolation purposes were of several kinds, as follows:

(1) Kraft grocery bags: 12-, 4-, and 2-pound sizes.
(2) Hemp-paper bottomless bags: 12-, 8-, and 4-pound sizes.
(3) Vegetable-parchment bags, large size: Flat bags, approximately 5½ by 17 inches in size, were made by hand from 30-pound parchment paper, waterproof glue being used for the seams.
(4) Vegetable-parchment bags, medium size: 40-pound parchment, gusset style, factory made, 4 by 2½ by 11¼ inches in size.
(5) Vegetable-parchment bags, small size: 40-pound parchment, gusset style, factory made, 3 by 2 by 9 inches in size.
(6) Cellophane bags: Similar in size and construction to the large-size parchment bag, but made of clear cellophane.

FIGURE 1.—General view of the selfing plot where the different types of bags were used.

So far as possible each type and size of bag was used on every beet plant in the test. A general view of the plot used for the bagging studies is shown in figure 1.

In addition, cloth cages of the type employed by Vilmorin were used on some 70 plants. These cages were constructed from a closely woven heavy cotton cloth designated by the manufacturer as 37-inch width, 2:35 yard extra high count drill. The material was preshrunk in a laundry before the cages were constructed. Two widths of material were sewed together lengthwise to form a cylindrical cage, the top being so cut that it could be closed with four seams, as shown by the end view in figure 2. A heavy snap attached to the top of the bag was snapped to a no. 9 wire fastened to the tops of posts, three cages being suspended from the wire between two posts (fig. 3). Two wire

hoops, made of no. 8 wire, the ends of which were spot-welded together, were used to keep the cage open (fig. 2). They were held in place by ⅝-inch tape which was sewed at four places, equal distances apart, to the inside of the cage. The lower hoop was placed 18 inches from the bottom of the cage, while the upper one was placed at the point where the top seams began. Two cages were made up with Cello-Glass fronts.

The posts and wire were set up before the bagging season. Three stakes were driven into the ground at equal distances apart around the plant. A galvanized-iron hoop, 12 inches in diameter, was constructed from a band of material 4 inches in width, with the upper edge turned outward. It was placed over the plant and forced into the ground an inch or more. The cage was very quickly placed in position by attaching the snap to the wire, tying the three pieces of outside tape to the three stakes, and drawing the draw-string up tightly about the metal hoop.

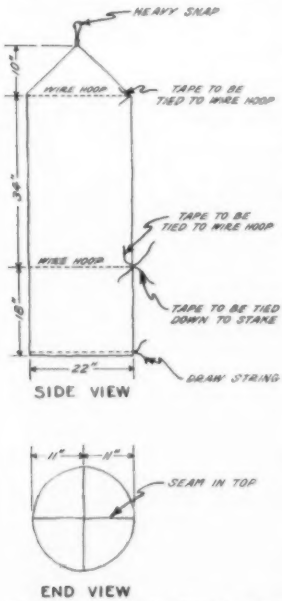

FIGURE 2.—Sketch showing side view and end view of cage.

In conducting the caging experiment, several red beets were included in the planting and all the beets left to flower were caged. There was little opportunity, under these conditions, for stray pollen to be blown out of one cage and into another, and insects appeared to be effectively excluded. This plan is similar to one used with sweetclover by L. E. Kirk, at the University of Saskatchewan, Saskatoon, Canada.

As a check on the efficiency of the methods of excluding foreign pollen, red beets were also planted at regular intervals in the plot where the bagging studies were conducted. Since any off-pollinations of sugar beets with red beets result in a red F_1 hybrid, it is apparent that this method would afford a test of the effectiveness of the method of isolation.

The branches enclosed in bags were, for the most part, the upper portions of the primary flowering stalks. About the same amount of flowering stalk was enclosed in each bag, except in the 2-pound size; in the latter it was usually necessary to enclose a smaller part of the inflorescence.

The relative humidity of the air enclosed in the various bags and cages was determined by circulating the air through a Selsi precision hair hygrometer in a closed circuit. A hand bicycle pump was used to exhaust the air from the bag and force it over the hygrometer. The screw cap on top of the pump was equipped with airtight packing and an outlet copper tube was brazed into this cap. The hygrometer was enclosed in a galvanized-iron box with a glass front that was soldered and sealed airtight, except the points of connection. This box was connected in the circuit with the pump and bag or cage and the air was circulated through the apparatus (fig. 4). Twenty

strokes of the pump were used for the set-up with each bag. The rubber tube connection into the hygrometer and out of the pump on

FIGURE 3.—View of caging plot in which individual plants were enclosed in single muslin cages.

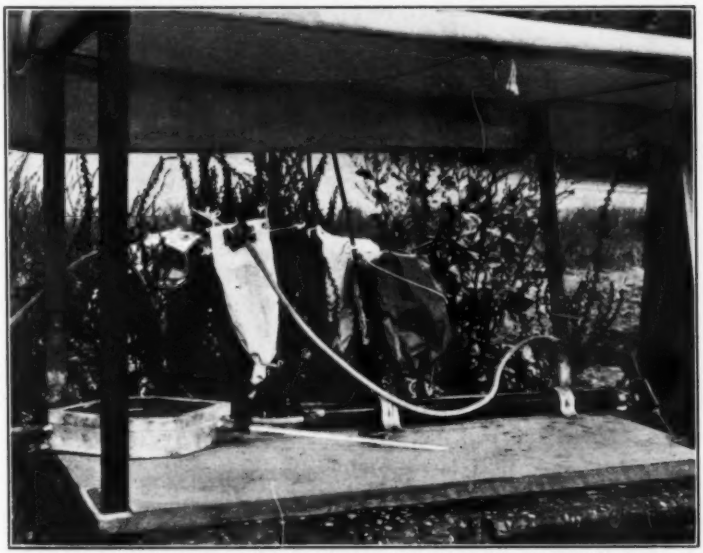

FIGURE 4.—Apparatus for determination of relative humidity. It consists of a hand bicycle pump and hygrometer connected into a closed circuit with a vegetable-parchment bag on a sugar-beet plant.

the return to the bag was then closed with clips and the apparatus connected up with the same type of bag on another plant under the

same type of shade. The total increase on the hygrometer for the two bags was read and recorded.

After the hygrometer was read it was set aside and not used again for 20 minutes or more. After each set a few strokes changed the air in the pump. Six hygrometers were provided for this purpose and used in rotation, but randomized as to the order. Six check readings on the hygrometers were taken before starting the experiment.

TABLE 1.—*Check readings on Selsi hair hygrometers*

Hygrometer no.	Reading no.						Mean	Hygrometer no.	Reading no.						Mean
	1	2	3	4	5	6			1	2	3	4	5	6	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	48	52	64	79	65	51	60	4	44	48	58	72	59	44	54
2	48	50	65	83	66	51	62	5	48	54	62	72	62	48	58
3	48	53	61	73	65	47	58	6	45	52	58	77	60	47	57

It will be observed from these check readings (table 1) that hygrometer no. 2 appears to average slightly higher and no. 4 slightly lower than the others, but the differences are fairly consistent for the six readings. For this particular comparative purpose, these hair hygrometers appear to be very practical. The results obtained seem to be sufficiently accurate for the requirements of this experiment.

In an attempt to obtain readings on the temperature of the air enclosed in the bag a thermometer (fig. 4) was inserted through a rubber cork into the galvanized-iron box containing the hygrometer. Readings taken in this manner did not indicate any material difference between the various bags. A later attempt to obtain accurate readings by inserting the bulb of a thermometer into the bag showed striking differences between bags. Data for the latter method only will be presented. It is probable that as the air was pumped through the apparatus any differences in the temperature of the air among the different bags were neutralized by the hygrometer housing and the pump with its connections.

EXPERIMENTAL RESULTS

RELATIVE HUMIDITY WITHIN BAGS AND CAGES

The relative humidity readings were taken (table 2) in order to compare the different types of bags. For these comparisons, the readings on all bags under a particular type of shade were taken as rapidly as possible, the time consumed for a particular set of bags never exceeding 1 hour. In these comparisons there was an unavoidable error as the air contained in the small bag was diluted to a much greater extent by the air contained in the pump and connections than was the air in a large bag. The branches contained in the small bags, however, were usually similar in size to those enclosed in the large bags.

Table 2 shows that the brown bags, either kraft or hemp, were consistently lower in relative humidity than were the vegetable parchment bags. The muslin cage gave results similar to those of the brown bags. The cellophane bags are comparable in size to the large parchment type, but gave slightly lower average tests for humidity, the difference being of doubtful significance. With but one exception higher humidity values were obtained in medium and small bags constructed of 40-pound parchment than were obtained with the large bags constructed of 30-pound parchment.

TABLE 2.—*Increase in hygrometer readings, resulting from circulating air from 2 bags or from a muslin cage through the hygrometer*

TABLE 2.—*Increase in hygrometer readings, resulting from circulating air from 2 bags or from a muslin cage through the hygrometer*

[The comparisons are for different bags on the same plants]

Date (1931)	Hour [a]	Weather conditions			Type of shade	Results for different types of isolators									
						Kraft bags			Hemp bags		Vegetable-parchment bags			Cello-phane bags	Muslin cages
		Wind	Clouds	Temperature (° C.)		12-pound	4-pound	2-pound	12-pound	4-pound	Large	Medium	Small		
July 2	10:00 a.m.	Heavy	Slight	30	Lath	4	5	3			9	14	17	18	11
	8:15 a.m.	do	Clear	28	None	7	7	8			8	41	12	14	4
	11:10 a.m.	do	Slight	30	Tobacco cloth	2	5	13			19	21	16	24	16
	1:10 p.m.	do	do	31	Cheesecloth	6	4	7			8	20	15	17	7
	2:25 p.m.	do	Clear	31	Lath	4	4	8			23	17	20	17	4
	2:30 p.m.	do	do	31	None	9	13	6			25	37	39	18	3
July 3	10:30 a.m.	Slight	Slight	32	Lath	3	1	6			12	15	10	11	14
	6:10 p.m.		Clear	23	None	18	13	5			9	18	12	20	6
	11:00 a.m.	Medium	Slight	33	Tobacco cloth	3	7	7			14	12	20	19	15
	9:45 a.m.	do		29	Cheesecloth	1	1	5			8	7	15	7	16
	3:55 p.m.	do	Heavy	32	Lath	5	2	1			2	7	18	8	15
	3:15 p.m.	do	do	33	None	3	4	7			6	10	10	10	6
July 5	10:15 a.m.	Slight to medium	Clear	28	Lath	2	3	6	2	2	14	24	15	16	
	10:50 a.m.	do	do	30	Tobacco cloth	2	5	8	2	3	13	12	22	33	
July 6	8:30 a.m.	Slight	do		None	3	4	6	2	4	24	25	35	18	13
	9:55 a.m.	do	do		do	4	8	10	6	2	20	21	30	12	9
	10:40 a.m.	do	do		do	7	10	11	4	4	20	24	26	12	6
	1:15 p.m.	do	do		do	12	8	11	6	3	24	25	19	17	5
	2:25 p.m.	do	do		do	9	10	9	7	3	40	20	32	15	7
	3:30 p.m.	do	do		do	16	12	7	4	6	29	17	19	27	10
	4:40 p.m.	do	do		do	10	6	7	5	5	16	22	18	16	7
July 7	7:15 a.m.	do	do		do	9	8	6	4	3	27	25	24	19	4
Mean of all						6.3	6.4	7.1			16.8	19.7	20.2	16.7	
Mean of July 5 and 6						7.4	7.4	8.1	4.2	3.5	22.7	21.5	24.0	18.5	
Mean of all but July 5						6.8	6.6	7.2			17.2	19.9	20.4	16.0	8.0

[a] When the comparisons were started. From 25 minutes to 1 hour was required to complete the readings on 1 series.

TABLE 3.—*Increase in hygrometer readings, resulting from circulating air from 2 bags or from a muslin cage through the hygrometer*

[The comparisons are for different types of shading]

Date (1931)	Hour	Weather conditions	Type of shade	Results for different types of isolator									
				Kraft bags			Hemp bags		Vegetable-parchment bags			Cellophane bags	Muslin cage
				12-pound	4-pound	2-pound	12-pound	4-pound	Large	Medium	Small		
July 4	9:40 to 11:45 a.m.	Few clouds; 25° C.; windy	None	10	5	5	3	2	24	30	17	18	2
	Do	do	Lath	2	3	4	2	2	15	24	19	13	9
	Do	do	Tobacco cloth	4	4	7	4	0	15	15	16	24	2
5	11:25 to 12:35 a.m.	Clear; 32° C.; slight breeze	None	12	12	4	5	4	24	27	32	15	5
	Do	do	Lath	3	3	5	2	1	10	30	16	15	7
6	8:50 to 9:55 a.m.	Clear; 25° C.; slight breeze	None	16	8	10			18	43	26	17	12
	Do	do	Cheesecloth	5	4	1			13	11	17	22	9
7	8:20 to 9:35 a.m.	Clear; 20° C.; slight breeze	None	11	13	8	4	3	17	24	28	23	4
	Do	do	Tobacco cloth	5	3	13	5	7	10	10	14	13	10

For the shading comparisons (fig. 5) the readings on a particular bag and shade were taken and followed immediately by readings on the same type of bag without shade. The data obtained for this comparative study are summarized in table 3.

It was possible to make paired comparisons by Student's method (*1*) to determine the significance of the differences between the humidity values obtained for no shade (check) and for lath, cheesecloth, or tobacco-cloth shading. Table 4 shows results obtained for these comparisons, based on Fisher's T test (*6*) for significance. While shading appears to result in somewhat lower average humidity values for each of these comparisons, only the mean of the lath versus check

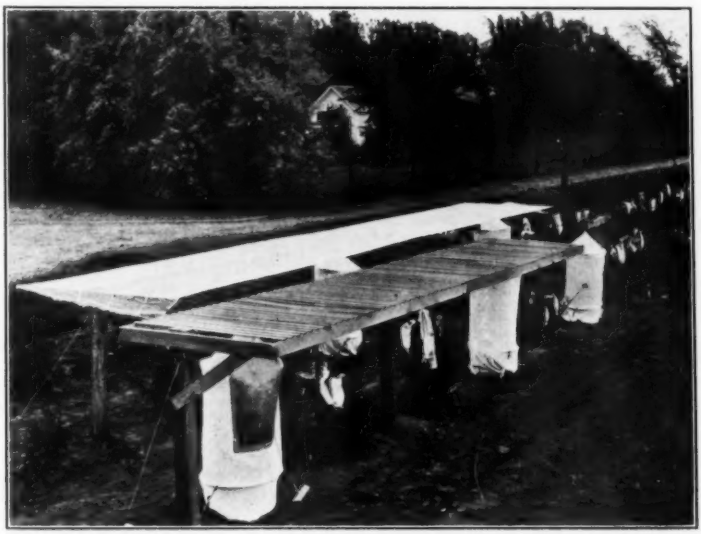

FIGURE 5.—View of plot used for relative-humidity and temperature determinations. In the foreground is the lath shading; in the background, the cheesecloth and tobacco-cloth shading.

readings for July 4 and 5 gives a P value less than 0.05, the difference required for the 5-percent level of significance.

TABLE 4.—*Paired comparisons of relative humidity values according to Student's method*

Date (1931)	Shade comparisons [a]	P
July 4	Check with lath	Between 0.2 and 0.1
July 5		Between .1 and .05
Mean		Between .05 and .02
July 4	Check with tobacco cloth	Between .3 and .2
July 7		Between .2 and .1
Mean		Between .1 and .05
July 6	Check with cheesecloth	Between .1 and .05

[a] Paired comparisons were made by Student's method (*1*) to determine the significance of the differences between the humidity values obtained for no shade and for lath, cheesecloth, or tobacco-cloth shading.

TEMPERATURE WITHIN BAGS AND CAGES

A small hole was made in each bag and the bulb of a thermometer inserted inside, the thermometer being attached to a bamboo stake. A different thermometer was used for each bag. The bags and cages were unshaded. Readings were made once each hour. A shaded, uncovered thermometer was used as a check. The summarized data are presented in table 5.

TABLE 5.—*Temperature readings with the thermometer bulb inside the bag or cage, as compared with check readings on a shaded uncovered thermometer, July 7, 1931* [a]

Hour [b]	Check	Temperature (°C.) within type of isolator indicated									
		Kraft bags			Hemp bags		Vegetable-parchment bags			Cellophane bags	Muslin cage
		12-pound	4-pound	2-pound	12-pound	4-pound	Large	Medium	Small		
8 a.m.	19.6	27.3	32.9	25.6	33.1	29.4	27.1	25.5	25.4	23.1	23.9
9 a.m.	20.6	28.8	30.9	27.6	34.1	30.9	29.6	27.0	26.4	27.1	25.9
10 a.m.	20.6	31.8	30.4	29.6	36.1	30.4	31.6	27.5	26.9	28.1	27.9
11 a.m.	21.6	33.8	28.9	30.6	35.6	28.9	32.1	28.0	26.9	28.1	29.4
12 a.m.	23.6	35.3	28.9	30.1	34.6	26.9	30.1	27.5	25.9	27.6	29.9
1 p.m.	26.1	35.3	28.9	28.6	33.1	28.4	29.1	28.5	26.9	30.1	30.9
2 p.m.	27.1	34.8	29.9	30.6	30.1	30.4	28.1	29.0	28.4	31.6	32.4
3 p.m.	28.1	32.8	30.4	31.6	30.1	31.4	27.6	30.5	28.4	32.6	32.4
4 p.m.	28.1	28.8	31.4	31.6	30.1	32.4	27.1	30.5	26.4	32.6	31.4
5 p.m.	28.1	28.3	30.9	30.6	30.6	31.9	28.1	31.0	25.9	32.6	28.9
Mean temperature	24.4	31.7	30.4	29.7	32.8	30.1	29.1	28.5	26.8	29.4	29.3

[a] All bags were on the same plant, and the muslin cage was on an adjacent plant. The bagged plant had flowered to the extent of about 75 percent. The values given in the table have been corrected in relation to a standard, so that they represent actual comparable temperatures for the various treatments.
[b] Brilliantly clear all day; slight to medium breeze.

The differences in temperature within the various bags were not large. However, the temperatures in all the bags showed some increase over the temperature of the exposed check, and the temperatures within the kraft and hemp bags were higher than those within the vegetable-parchment and cellophane bags or the muslin cage.

SEED SETTING

The summarized data for the bagging and caging studies are presented in tables 6 and 7. Seed counts were made after the seed balls had been cleaned by use of a draper and hand sieves. The seed balls appeared to have good germs.

TABLE 6.—*Percentage of plants setting seed under different types of bags*

Type of plant	Percentage of plants setting seed in type of bag indicated									
	Hemp			Kraft			Vegetable parchment			Cellophane
	12-pound	8-pound	4-pound	12-pound	4-pound	2-pound	Large	Medium	Small	
Commercial variety	42	48	30	50	38	30	87	8	7	17
Some inbreeding	0	50	.....	74	44	38	95	15	6	0

TABLE 7.—*Seed setting obtained for all bagged and caged plants*

Seed produced per plant	Bagged plants				Caged plants			
	Commercial variety		Some inbreeding		Commercial variety		Some inbreeding	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
None	96	47.5	8	25.8	5	11.4	1	25.0
Less than 30 seeds	53	26.2	8	25.8	12	27.3	1	25.0
From 30 to 99 seeds	25	12.4	4	12.9	6	13.6	0	
100 or more seeds	28	13.9	11	35.5	21	47.7	2	50.0
Total	202	100.0	31	100.0	44	100.0	4	100.0

On commercial varieties, from 30 to 99 seed balls per plant were obtained on 12.4 percent of the bagged plants and on 13.6 percent of the caged plants. One hundred or more seed balls per plant were obtained on 13.9 percent of the bagged plants and on 47.7 percent of the caged plants. Some seed balls were obtained on 52.5 percent of all bagged plants and on 88.6 percent of all caged plants. A much higher percentage (74.2) of seed setting was obtained on the bagged plants that had some inbreeding. This difference indicates that selection in inbred lines tended to isolate material which was genetically more capable of producing seed through forced self-fertilization. It is further supported by the fact that certain plants in both groups appeared to set seed under different types of bags and cages much more freely than others (fig. 6). A similar conclusion was reached by Nilsson (*8*).

In order to prevent the entrance of thrips or other small insects cotton was used about most of the stems of branches enclosed in bags. The mouth of the bag was then tied firmly about the cotton. All the bags on 19 plants were purposely left without cotton. Table 8 shows a comparison between seed setting following the use of cotton about the stems and seed setting when cotton was not used. All bags on the plants regardless of type were considered in this study.

TABLE 8.—*Seed setting with and without the use of cotton*

Treatment	Total plants	Plants setting some seed		Plants not setting seed	
	Number	*Number*	*Percent*	*Number*	*Percent*
With cotton	172	80	47	92	53
Without cotton	19	7	37	12	63

Of the plants bagged without cotton about the stems, 37 percent set some seed as contrasted with 47 percent for those bagged with cotton. Had insects entered where the cotton was lacking, quite the opposite result would have been expected. The fact that a markedly higher percentage of seed setting did not result from failure to use cotton seems to indicate that any insects that might have entered the bag where cotton was not used did not constitute an effective means of pollen distribution between plants under the conditions of this test.

A germination test and a seedling study were made on the seed balls from plants which set upwards of 100 or more seed balls. These results are summarized by classes in table 9.

The results obtained show fairly well the efficiency of the methods used to exclude foreign pollen and limit the pollination among the flowers inbred. From a total of 1,350 seed balls, representing 50 each for 27 bagged plants, 721 seedlings were obtained. Not a single plant appeared to be a hybrid with a red beet. Cotton was used

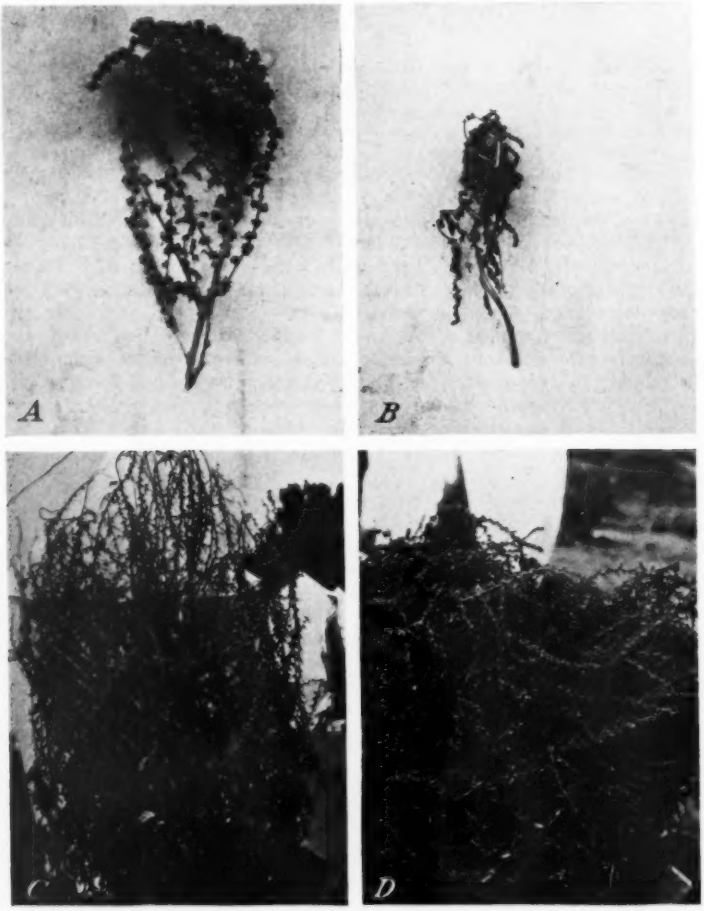

FIGURE 6.—*A*, Branch that when covered with a 4-pound hemp-paper bag produced more than 400 good seed balls; *B*, branch that when covered with a cellophane bag produced no seed; *C*, close-up showing seed on plant that produced an excellent crop of seed under a cage; *D*, close-up of plant showing wealth of inflorescence but practically no seed produced under a cage.

about the stalks for all the 27 bagged plants represented. Out of 269 seedlings obtained from 690 seed balls from 23 caged plants, 2 seedlings appeared to show all the characteristics of F_1 crosses with red beets. While these may have been due to foreign pollen carried out of one cage and into another by wind or insects, it is believed more likely that they resulted from cross pollination before the cages were

placed in position. The first florets to open are those situated low on the main stalk or in the axils of the branches and are easily overlooked, even after very careful examination. The cages were placed in position on each plant only a day or two before the opening of the earliest flowers on the plant.

TABLE 9.—*Summary of greenhouse germination test on selfed seed*

[Fifty seed balls each were used from bagged plants and 30 each from caged plants]

Type of isolator	Number of plants falling in indicated class based on number of seedlings per 100 seed balls										Mean germination
	0	1–20	21–40	41–60	61–80	81–100	101–120	121–140	141–160	Total	
											Percent
Bags	1	7	5	1	6	4	1	1	1	27	39.0
Cages	3	8	1	4	4	3				23	39.0

The mean germination percentage was 39.0 for seed from both bags and cages. About 50 percent of the plants fell in classes ranging from 41 to 160 seedlings per 100 seed balls. By planting rather heavily, it would be possible to carry as breeding material nearly all the progeny lines represented in table 9. From the practical standpoint, however, assuming poor germination to be possibly heritable, such lines would be discarded.

Of the several types of bags used in the study (table 6), the large vegetable-parchment bags appeared to give the best results, and the medium and small parchment bags proved to be the most unsatisfactory. The kraft and hemp bags were about equally desirable. Of 30 cellophane bags used on 29 plants, seed was obtained on 5 plants, only 2 of which gave more than 5 seed. These 2 yielded 105 and 120 seed, respectively. It is very likely that these seed may have been the result of off-pollination, since the glue used did not hold satisfactorily on the cellophane bags. At harvest, these bags were carefully checked and those showing a loose seam were discarded. In the few exceptional cases a faulty seam may have escaped notice.

Under the conditions of this experiment, the differences in temperature and relative humidity of the air contained in the bags and cages appeared to be of doubtful value in explaining the differences in seed setting. The use of brown bags, both kraft and hemp, resulted in low humidity and high temperature within the bag, but rather good seed setting. The use of parchment and cellophane bags resulted in comparatively high humidity and low temperature, and low seed setting, with the exception of the large parchment bags, the use of which resulted in the highest seed setting of all the bags tested.

Cello-Glass, which was very easily sewed directly to the heavy drill cloth, was used in the construction of two cages (fig. 5). The plants appeared to grow quite normally inside these cages, just as they did in many of the regular cages. It is doubtful whether the use of Cello-Glass windows in the cages was of any value under the conditions of this experiment. Owing to the extreme genetic variability among the mother beets used for these studies, the seed setting on these two plants is of no value in estimating the efficiency of Cello-Glass windows for cages.

One plant was cut back to a single main stalk and a cage was used to cover only the stalk. The foliage about the crown was not covered and appeared quite normal throughout the season. The seed setting on this plant was very poor.

DISCUSSION

In the absence of pertinent genetic data, it may be assumed, for theoretical purposes, that the ability to produce seed under conditions of forced self-fertilization is hereditary in nature. The data obtained in this study tend to support this theory. In ability to produce viable seed under bags or a cage, there were striking differences among plants obtained from commercial varieties; and the plants with some inbreeding were capable of producing more selfed seed than the selections from commercial varieties.

With regard to seed setting when plants are bagged, the results obtained in this study on sugar beets are very similar to those obtained in studies on rye by Heribert-Nilsson (*7*) and Brewbaker (*4*). The conclusion was reached by the latter that the characters of high and low self-fertility appear to be heritable and that by selection and continued self-pollination highly self-fertile lines can be obtained.

Assuming also that there are genetic factors conditioning the viability of the seed produced, it is to be confidently expected that by continued selfing and selection in self-fertilized lines the strains isolated should become relatively more easy to continue, as the poorer seed producers and factors for low viability are discarded. This will be all the more true if, as Nilsson (*8*) found for certain lines, appreciable loss in vigor results from inbreeding.

SUMMARY AND CONCLUSIONS

A study was made of the relative effect of various factors on seed setting in sugar beets under conditions of forced self-fertilization.

Ten different types of bags were used. So far as possible, each type of bag was placed on every plant. A large hand-made bag, about 5½ by 17 inches, constructed of 30-pound vegetable parchment, gave the most promising results, 87 percent of the bags containing some seed. The 12-pound and 8-pound hemp and the 12-pound kraft bags contained some seed on 42, 48, and 50 percent, respectively, of the plants so bagged. The 4-pound hemp and 4-pound and 2-pound kraft bags were next in order of promise, while plants enclosed in factory-made medium bags and in small-size parchment and cellophane bags yielded very poor seed setting. For plants with some inbreeding the seed setting was somewhat higher in each case, but the comparative values were similar.

A specially constructed muslin cage was used in an isolated plot, all plants in the plot being caged. Of about 44 plants caged, only 11.4 percent failed to set seed, the others ranging from poor to practically perfect seed setting. This difference was, in all probability, hereditary in nature.

Wide differences were obtained in the relative humidity of the air exhausted from the different bags. The relative humidity for the large parchment bag appears to have been about the same as that for the medium and small parchment bags, but was much higher than

for the brown hemp or kraft bags. The differences in this respect do not correlate with differences obtained in seed setting.

When the sun was shining, the temperature within the brown bags was higher than it was within the white bags. There was no correlation in this respect with the differences in seed setting

Two specially constructed cages having Cello-Glass fronts were used in the experiments. In another instance, a plant was entirely enclosed in a cage, with the exception of the crown leaves. No particular advantage appeared to result from the use of either of these special methods.

The results obtained indicate that some method of isolation in which either bags or cages are used may prove entirely practicable in intensive breeding and genetic work with sugar beets.

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MAIZE CROSSING VALUES IN SECOND-GENERATION LINES[1]

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INTRODUCTION

Puerto Rican farmers are unfamiliar with the use of maize lines inbred through many generations for the production of first-generation hybrid seed. The aim of the breeding program of the Federal Agricultural Experiment Station at Mayaguez has been to test crossing values of lines inbred through the relatively short period of 2 or 3 generations; it was thought that their reasonably good productivity would facilitate their use under local conditions. Two methods have been devised for such tests, line-variety and line-recessive yield trials. The hybrids used in the line-recessive yield trials were produced by pollinating the more vigorous lines with a line assumed to be recessive because of its white kernel color and inferiority in plant size, ear size, and yield. The object of the work described in this paper was to determine whether increased yields could be secured by outcrossing lines inbred only two generations and whether the crossing values of these lines could be tested by either of the foregoing methods.

REVIEW OF LITERATURE

No data have been noted in the literature covering the use of the line-recessive method. Jones[3] reported on line-variety hybrids in 1921 though not as a method of testing lines. Yield trials with line-variety crosses and their suggested use as a basis for elimination in selfed lines were briefly reported on in January 1929 by the Mayaguez Station.[4] Since that is thought to be the first published suggestion of the use of this method and in view of the marked interest shown at present on line varieties this report is quoted in full as follows:

> Crosses were made between a common open-pollinated variety of native corn and 27 lines of corn originating from high-yielding Porto Rican parent ears. These lines had only been self-pollinated twice, but it was thought that the best lines for additional selfing could be determined by comparing the yields of the hybrids with the yields of a common pollinator. Most of the hybrids were expected to equal and a few slightly to exceed in yield the ordinary native corn, which was used as the pollen parent. The results were very encouraging. The average yield of the hybrids was approximately 20 percent greater than that of the check variety or pollen parent. Taken as a whole, the ears of the hybrids had very sound grain, and were much freer from mould and from worm injury than the ears of the check. The hybrid plants were sturdy and lodged less frequently than did those of the check variety.

Lindstrom[5] reported significant increases in yield over open-pollinated parent varieties through the use of line-variety crosses.

[1] Received for publication Apr. 19, 1933, issued April 1934.

[2] Acknowledgment is made to Alberto Correa, of the Insular Department of Agriculture and Commerce, for cooperation in the tests made at Utuado; and to Guillermo Cabrera, of Central Coloso, and Luis Serrano, of the Insular Experiment Station, for cooperation in seed production.

[3] JONES, D. F. THE PRODUCTIVENESS OF SINGLE AND DOUBLE FIRST GENERATION CORN HYBRIDS. Jour. Amer. Soc. Agron. 14: 246. 1922.

[4] DAVIS, R. L. REPORT OF THE PLANT BREEDER. P.R. Agr. Expt. Sta. Ann. Rpt. 1927: 14–15. 1929.

[5] LINDSTROM, E. W. PREPOTENCY OF INBRED SIRES ON COMMERCIAL VARIETIES OF MAIZE. Jour. Amer. Soc. Agron. 23: 652-661, illus. 1931.

Jenkins and Brunson [6] found significant correlations between yields of inbred lines crossed inter se and the same lines in line-variety combination; they concluded that crosses with open-pollinated varieties may be used efficiently in preliminary testing of new lines.

The data presented in this paper differ from those already published in full by Lindstrom, and Jenkins and Brunson, in that they are based on less homozygous material, only two generations of inbreeding being practiced prior to crossing. Most of the parent-progeny correlation studies reported on in the literature are on lines inbred from 5 to 6 generations, but Jenkins [7] reported on less homozygous material and found high-yielding values in inbred lines in their F_1 crosses indicating that even after only 3 or 4 generations of selfing lines must be homozygous for many of the factors that make for yield. He reports also significant and positive correlations between yield of the F_1 crosses and most of the characters in the inbred line that make for plant vigor; these correlations indicated that the most productive crosses may be expected from the most productive inbred parents.

METHODS OF EXPERIMENTATION

The maize used for breeding material was collected from many districts in Puerto Rico, and the less desirable ears were eliminated. The foliage weight of 20 to 25 seedlings at 3 weeks was compared with the weights of other cultures grown in the same germination flat, and parent ears were retained which produced large dark-green seedlings. The highest yielding parent ear from each district as determined in hill-checked ear-row trials was inbred. The process of selection in selfed lines was essentially the same. Lines were favored which compared well with open-pollinated corn in plant growth.

Since a similar reaction or parallel performance among related lines in hybrid combination would afford strong evidence that early elimination is justifiable, the inbred material tested comprised a number of groups of sibs. In order to include a wide range of breeding stock, lines were chosen from unrelated parent ears each one of which originated in a different district. Thus, although only 51 lines are reported on, containing 10 groups of second-generation sibs, they were derived from 8 parent ears entirely unrelated to each other. Taken as a whole, they are fairly representative of the corn for the entire island.

When elimination is practiced early in the process of selfing and the aim is to retain only high yielding lines with large plant growth, it is particularly important to guard against contamination during hand pollination, as hybrid plants are easily confused with selfs. The bag-withdrawal method was devised for this purpose. The well-known bottle method was used, but instead of removing the ear bag upward, as is customary, thus tending to expose the silks, it was ripped open and pulled straight downward after the tassel bag and the tassel were in position. The use of the bag-withdrawal method enables the worker to remain 4 to 6 inches from the silks and safeguards them against being touched by the hand and against contamination with foreign pollen.

[6] JENKINS, M. T., and BRUNSON, A. M. METHODS OF TESTING INBRED LINES OF MAIZE IN CROSSBRED COMBINATIONS. Jour. Amer. Soc. Agron. 24: 523–530. 1932.

[7] JENKINS, M. T. CORRELATION STUDIES WITH INBRED LINES AND CROSSBRED STRAINS OF MAIZE. Jour. Agr. Research 39: 677–721. 1929.

In producing the hybrid seed it has been the practice when detasseling to destroy the short plants which are easily overlooked and might otherwise shed contaminating pollen. Late-flowering plants of the pistillate parents were topped just above the upper ear in order to reduce supervision and again to lessen the chance of contamination; the cost of hybrid-seed production is also reduced by this operation as some plants may be 6 to 8 days late in tasseling and when left nearly double the detasseling period.

In all the yield trials reported herein a control or check variety was planted in every other or every third plot. The plots were long and narrow, either 1 or 2 rows wide. It is thought that the direct comparisons on yields with those of adjoining checks plots were fairly reliable and that the correction for variations in drainage and soil moisture thus secured more than offset the effect of intervarietal competition.

For determining the significance of yield differences Love's modification of Students' method was used.[8]

EXPERIMENTAL DATA

YAUCO-TORRE LINE-VARIETY YIELD TRIALS, MAYAGUEZ, 1927

Yauco-Torre corn, a completely heterozygous open-pollinated variety, and 27 line-variety hybrids produced at Mayaguez in 1926 were used in an experiment to determine the yields of second-generation inbred lines when top crossed by Yauco-Torre. The staminate parent was an open-pollinated variety from Yauco. The pistillate parents were 27 second-generation lines derived from 3 high-yielding parent ears from Lajas, Peñuelas, and Jayuya. The seed was stored in sealed containers with quicklime during the winter season. Each hybrid consisted of seed from 35 to 50 ears bulked together.

Planting was heavy, 4 to 5 seeds per hill. The stand was thinned to 1 plant per hill and consisted of 10,900 plants per acre. Each 1-row plot consisted of 24 plants and was one five-hundredth of an acre in area. Every other row was planted to the check variety. A very heavy wind and rain storm toward the end of the crop season caused considerable lodging in the check corn but did little damage to the hybrids; as a result there was somewhat more decay in the check than in the hybrid ears.

Four plots were grown. In plot E each variety was planted in a single row and compared directly with an adjoining row of the Yauco-Torre corn. In plot F each variety was repeated, but no checks were grown on account of the limited area of land available. In plots K and L each line variety was grown once in comparison with adjoining check rows of Castillear-1-5-1 × Yauco-Torre, one of the more promising hybrids. In plot E there were 26 replications of the Yauco-Torre check corn. In plots K and L the line-variety check was replicated 52 times.

Yields per acre of the various plots are given in table 1 in bushels of air-dry shelled corn. The moisture content of the various hybrids was assumed to be the same as none of the corns was contrasting in kernel type. This procedure probably gave a slight advantage to the Yauco-Torre corn which was in general a somewhat softer type of corn and consequently higher in moisture content.

[8] LOVE, H. H. A MODIFICATION OF "STUDENTS" METHOD FOR USE IN INTERPRETING RESULTS. Jour. Amer. Soc. Agron. 16: 68–73. 1924.

TABLE 1.—*Yields of air-dry shelled corn (bushels per acre) in the Yauco-Torre line variety-hybrid trials, 1927*

Hybrid	Plot E			Plot F	Plot K			Plot L			Average increase over check in plots K and L	Average of 4 plots of hybrids
	Hybrid	Average of 3 or 4 nearest Yauco-Torre check rows	Increase(+) or decrease (−) as compared with check	Hybrid	Hybrid	Average of 3 nearest Castillear-1-5-1×Yauco-Torre check rows	Increase(+) or decrease (−) as compared with check	Hybrid	Average of 3 nearest check rows of Castillear-1-5-2×Yauco-Torre	Increase(+) or decrease (−) as compared with check		
Castillear-1-5-1×Yauco-Torre	63.5	47.5	+16.00	54.95								
Castillear-1-5-2×Yauco-Torre	43.8	36.9	+6.90	46.50	54.9	48.83	+6.07	33.8	47.55	−13.75	−3.84	44.80
Castillear-1-3-1×Yauco-Torre	38.1	33.23	+4.87	43.90	58.6	48.12	+10.48	45.3	43.65	+1.65	+6.07	46.48
Castillear-1-1-1×Yauco-Torre	56.5	32.80	+23.70	48.30	45.5	47.20	−1.70	40.0	47.90	−7.90	−4.80	47.60
Vicens Flint-2-2-2×Yauco-Torre	42.2	32.10	+10.10	35.70	40.0	47.51	−7.51	32.7	44.55	−11.85	−9.68	37.65
Vicens Flint-2-9-1×Yauco-Torre	37.5	26.33	+11.17	39.15	36.4	48.53	−12.13	34.8	45.10	−10.30	−11.21	36.96
Vicens-21-1-3×Yauco-Torre	40.1	26.7	+13.40	23.50	40.0	45.23	−5.23	40.3	43.30	−3.00	−4.11	35.97
Vicens Flint-2-14-2×Yauco-Torre	31.7	26.4	+5.30	31.20	38.6	45.50	−6.90	33.8	44.70	−10.90	−8.90	33.83
Vicens Flint-2-12-1×Yauco-Torre	49.6	27.1	+22.50	49.2	41.7	43.52	−1.82	35.9	42.15	−6.25	−4.03	44.10
Vicens Flint-2-4-1×Yauco-Torre	36.0	28.2	+7.80	57.0	42.2	44.75	−2.55	35.4	43.70	−8.30	−5.42	42.65
Cacique-1-2-1×Yauco-Torre	41.2	26.8	+14.40	53.15	44.9	42.80	+2.10	35.4	45.65	−10.25	−4.07	43.66
Cacique-1-2-4×Yauco-Torre	46.5	24.4	+22.10	48.65	54.9	45.40	+9.50	45.9	47.15	−1.25	+4.13	48.99
Cacique-1-50-1×Yauco-Torre	32.8	25.6	+7.20	35.65	30.6	45.5	−14.90	33.2	44.7	−11.50	−13.20	33.06
Cacique-1-67-1×Yauco-Torre	35.4	25.6	+9.80	47.80	41.7	49.55	−7.85	41.7	43.2	−1.50	−4.67	41.65
Castillear-1-4-1×Yauco-Torre	30.6	24.3	+6.30	45.45	32.8	46.50	−13.70	28.5	44.8	−16.30	−15.00	34.34
Castillear-1-4-3×Yauco-Torre	44.0	26.74	+17.26	59.60	42.8	46.5	−3.70	41.7	42.5	−.80	−2.25	47.03
Castillear-1-4-2×Yauco-Torre	31.2	29.70	+1.50	36.50	45.4	43.90	+1.50	32.7	44.85	−12.15	−5.32	36.45
Vicens Flint-2-9-2×Yauco-Torre	39.7	30.22	+9.48	48.00	42.2	41.00	+1.20	37.5	45.15	−7.65	−3.22	41.85
Cacique-1-78-2×Yauco-Torre	34.9	30.1	+4.80	36.15	41.2	41.0	+0.20	41.0	48.80	−7.80	−3.80	38.31
Castillear-1-3-4×Yauco-Torre	26.9	29.5	−2.60	44.10	58.6	41.6	+17.00	40.1	46.20	−6.10	+5.45	42.42
Castillear-1-3-2×Yauco-Torre	26.4	24.3	+2.10	54.00	46.5	44.5	+2.00	47.0	49.50	−2.50	−.25	43.47
Castillear-1-2-1×Yauco-Torre	46.5	26.4	+20.10	36.00	44.3	46.15	−1.85	48.7	50.85	−2.15	−2.00	43.88
Cacique-1-50-2×Yauco-Torre	23.8	22.54	+1.26	21.90	32.3	45.85	−13.55	41.5	52.70	−11.20	−12.37	29.87
Cacique-1-93-1×Yauco-Torre	21.7	18.76	+2.94	19.80	34.8	45.85	−11.05	45.3	52.6	−7.30	−9.17	30.40
Vicens Flint-2-3-1×Yauco-Torre	19.0	17.80	+1.20	37.80	55.2	43.60	+11.60	48.5	52.15	−3.65	+3.98	40.13
Castillear-1-3-3×Yauco-Torre	19.0	16.74	+2.26	40.10	43.3	41.90	+1.40	48.5	55.50	−7.00	−2.80	37.72
Marcucci Droop-10-1-1×Yauco-Torre	27.5	27.48	+.02	39.05	22.2	39.30	−17.10	27.4	52.30	−24.90	−21.00	29.04
Average	36.52	27.56	+8.96	41.97	42.75	45.00	−2.25	39.10	46.97	−7.87	−5.06	

Taken as a group the mean increase of the line-variety hybrids over the staminate parent variety in plot E was 8.96 bushels of air-dry shelled corn per acre (table 1). The 27 line-variety hybrids averaged 36.52 bushels per acre, while the Yauco-Torre check variety averaged only 27.56 bushels in an equal number of plots. The odds are 9,999 : 1 that this difference is significant.

The spacing and field conditions in plots K and L were the same as those in plot E. Instead of the Yauco-Torre corn being used as the check variety, Castillear-1-5-1 × Yauco-Torre, one of the hybrids which germinated well and whose inbred parent was outstanding for growth, vigor, and grain production, was chosen. The other 26 hybrids were inferior to those of the check in 38 of 52 comparisons. A general comparison was made between Castillear-1-5-1 × Yauco-Torre and the other 26 hybrids using the average yield of two plots of each variety as a unit. As an average for both plots K and L Castillear-1-5-1 × Yauco-Torre outyielded all the other hybrids by a margin of 5.06 bushels per acre. The odds are 4,999 : 1 that this is a significant difference.

PARALLEL PERFORMANCE IN SECOND-GENERATION SIBS

TABLE 2.—*Yields of air-dry shelled corn per acre from second-generation sib lines outcrossed with Yauco-Torre corn in the Yauco-Torre line-variety trials, Mayaguez, 1927*

First-generation inbred parent	Yields [a] of second-generation sib lines crossed with Yauco-Torre corn								Average shelled corn per acre yield [b] of sib crosses
	Shelled corn per acre	Proportion of Yauco-Torre yield	Shelled corn per acre	Proportion of Yauco-Torre yield	Shelled corn per acre	Proportion of Yauco-Torre yield	Shelled corn per acre	Proportion of Yauco-Torre yield	
	Bushels	*Percent*	*Bushels*	*Percent*	*Bushels*	*Percent*	*Bushels*	*Percent*	*Bushels*
Castillear-1-5	44.8	162.9	45.99	167.2					45.39±0.59
Castillear-1-3	46.4	168.6	43.47	157.8	42.44	154.1	37.72	137.0	42.51±2.42
Castillear-1-4	34.34	124.8	47.03	171.0	29.09	105.6			36.82±6.81
Vicens Flint-2-9	41.8	151.8	36.9	134.1					39.35±2.45
Cacique-1-2	43.66	158.7	48.99	177.9					46.33±2.67
Cacique-1-50	33.06	120.1	29.87	108.6					31.46±1.59
Yauco-Torre staminate parent	27.56								

[a] Each yield is that of a cross between a second-generation line derived from a first-generation line given in column 1 and Yauco-Torre corn and is the average of 4 plots.

[b] Averages of all sib crosses in 8 to 16 plots.

Comparison is made in table 2 between yields of six groups of second-generation sib lines crossed with Yauco-Torre corn and those of the Yauco-Torre staminate parent. The first-generation inbred-parent line of each group of sibs is given in column 1. In the case of each line-variety cross the average yield of 4 plots is given; the average yields of the sib groups given in the last column are those of 8 to 16 plots and that of the Yauco-Torre corn is the average of 27 plots. Groupings were made as follows: Very high yielders, the hybrids that exceeded the Yauco-Torre corn by 50 percent or over; high yielders, those that exceeded it by from 25 to 49 percent; and low yielders, those that exceeded it by less than 25 percent. Of 6 different groups of second-generation sibs lines, 5 gave a parallel performance, i.e., they were either all high yielders or all low or medium yielders when outcrossed with an open-pollinated variety.

The lines used in the line-variety crosses were largely derived from the three parent ears Castillear-1, Vicens Flint-2, and Cacique-1. Among 11 hybrids whose pistillate parents were Castillear-1 derivatives there were 8, or nearly three fourths, which were very high yielders, 1 high yielder, and 1 low yielder. Only half of the hybrids whose pistillate parents were derived from the other ears were very high yielders. Castillear-1 is outstanding as a source of desirable lines. This indicates the desirability of concentrating the work of self-pollination on a few high-yielding ear-to-row selections.

PARENT-PROGENY CORRELATION STUDIES, 1927 CROP

Correlation studies (see tabulation on p. 351) were made between line-variety yields and various characters of the inbred pistillate parents including yield, leaf width, seedling foliage weight, percentage of soft starch by weight, and the kernel type as indicated by the degree of denting. The average yield of the 4 plots of each hybrid in comparison with the general average of 31 plots of the Yauco-Torre check corn were used as the basis for the yield comparison. The yields were expressed in percentages of the check. In this way a comparison could be made between yields of the inbred lines in 1925 and 1926, and those of the hybrids in 1927. The seasonal variation from year to year makes a direct comparison in actual yields undependable. Tables 3 and 4 give the data for the years 1925 to 1927, inclusive, on both the inbred pistillate and the corresponding line-variety hybrids.

TABLE 3.—*Parent-progeny study of Yauco-Torre line varieties and inbred parents, 1925–27*

First inbred generation line	Shelled corn per acre							Ears per 100 bearing plants of S_2 line	Green foliage of S_2 parent lines at 3 weeks		Dry foliage of hybrids at 3 weeks		Weight of 100 kernels		Soft starch by weight
	First selfed generation		Second selfed generation, August 1926		Average yield of first and second inbred generations, proportion of check	Outcrossed with Yauco-Torre corn, July 1927			Weight	Proportion of Yauco-Torre check	Weight	Proportion of Yauco-Torre check [a]	S_2 lines	xenia generation	
	Yield	Proportion of check	Yield	Proportion of Yauco-Torre corn		Yield	Proportion of Yauco-Torre check								
	Bushels	*Percent*	*Bushels*	*Percent*	*Percent*	*Bushels*	*Percent*	*Number*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
Castillear-1-5-1	40.3	76.8	44.1	99.3	88.0	45.99	167.2	141.8	36.0	130.6	14.02	111.3	32.9	29.3	23.97
Castillear-1-5-2	40.3	76.8	45.7	103.1	89.9	44.8	162.9	142.1	31.5	95.7	13.82	109.8	35.1	28.6	
Castillear-1-1-1	51.2	94.3	26.4	59.7	77.0	47.6	173.1	101.8	28.7	91.6	16.59	106.0	37.4	30.5	27.39
Castillear-1-2-1	61.0	110.0	14.7	33.2	71.6	43.88	159.4	128.2	33.0	84.2	13.18	103.6	37.9	24.9	35.23
Castillear-1-3-1	66.5	145.1	24.4	54.9	100.0	46.41	168.6	152.7	44.2	136.6	18.81	143.7	47.3	32.2	22.48
Castillear-1-3-2	66.5	145.1	29.4	66.3	105.7	43.47	158.1	121.8	37.0	114.5	17.83	136.1	32.3	28.6	29.76
Castillear-1-3-3	66.5	145.1	12.1	27.3	86.2	37.72	137.1	126.7	28.9	82.5	14.53	106.8	27.6	23.7	18.90
Castillear-1-3-4	66.5	145.1	14.8	33.4	89.2	42.42	154.1	134.9	26.4	92.2	15.69	115.1	40.3	26.1	22.80
Castillear-1-4-1	43.3	74.7	21.2	47.9	61.3	34.34	124.8	171.3	20.9	66.7	11.30	75.3	27.8	24.0	20.19
Castillear-1-4-2	43.3	74.7	15.4	34.7	54.7	29.09	105.8	136.4	29.7	95.0	16.53	110.2	36.4	28.0	21.00
Castillear-1-4-3	43.3	74.7	17.9	40.3	57.5	47.03	171.0	125.0	37.4	115.6	14.90	112.4	33.3	24.2	21.86
Cacique-1-2-1	47.6	89.2	31.7	71.6	80.4	43.66	158.7	160.0	25.7	79.6	13.12	95.0	33.1	25.6	19.58
Cacique-1-2-4	47.6	89.2	25.3	57.1	73.1	48.99	177.9	134.0	22.9	70.8	14.11	102.1	22.7	28.6	16.86
Cacique-1-50-1	11.6	21.1	12.7	28.8	24.9	33.06	120.1	127.5	28.2	87.4	13.70	104.6	32.5	24.7	19.88
Cacique-1-50-2	11.6	21.1	5.2	11.8	16.4	29.87	108.7	125.5	20.6	63.8	11.02	84.2	30.4	26.4	25.10
Cacique-1-78-2	19.5	39.0	23.4	52.7	45.8	38.31	139.1	159.4	19.7	61.1	14.13	136.5	29.0	30.0	14.62
Cacique-1-67-1	7.3	13.2	27.3	61.7	37.4	41.65	151.4	131.2	20.9	64.2	12.56	103.2	28.8	25.6	26.11
Cacique-1-93-1	2.4	4.6	15.2	34.2	19.4	30.40	110.4	133.3	24.6	76.1	11.78	113.7	25.6	25.3	13.56
Vicens Flint-2-9-1	25.4	71.5	26.5	59.9	65.7	36.96	134.5	125.9	29.6	91.7	14.62	106.1	41.0	29.0	23.39
Vicens Flint-2-9-2	25.4	71.5	9.9	22.5	47.0	41.85	152.0	105.9	29.5	91.3	12.45	90.5	30.6	22.4	12.59
Vicens Flint-2-2-2	37.2	88.4	21.2	47.9	68.1	37.65	136.9	115.2	22.4	69.3	13.20	108.5	32.9	29.2	21.40
Vicens Flint-2-4-1	48.2	101.3	21.8	49.0	75.1	42.63	155.0	134.0	28.9	89.4	15.30	148.3	37.5	29.2	16.78
Vicens Flint-2-3-1	9.7	27.6	13.6	30.7	29.1	40.13	145.8	108.0	22.9	70.8	11.51	112.0	36.0	27.9	12.46
Vicens Flint-2-12-1	33.6	91.6	23.9	53.8	72.7	44.10	160.2	108.6	26.1	80.8	13.80	94.8	35.8	28.8	18.02
Vicens Flint-2-14-2	40.8	76.9	18.5	41.7	59.3	33.88	123.1	116.2	27.9	86.4	14.80	101.7	44.5	25.5	16.82
Vicens-21-1-3	48.1	12.9	10.9	24.6	18.8	35.97	130.7	132.6	30.8	95.5	14.76	111.3	38.0	27.7	28.52
Marcucci Droop-10-1-1	29.3	53.3	3.6	7.9	30.6	29.04	105.6	101.8	27.7	88.5		121.0	33.2		
Yauco-Torre corn, check			44.3			27.54			[b] 32.3						

[a] The dry foliage weights of the Yauco-Torre corn checks were variable in the different germination flats and are not given.
[b] This is the average of all checks; the percentages are based on individual check weights.

TABLE 4.—*Plant characters of first and second inbred generations of Yauco-Torre line variety pistillate parents, 1925–27*

Second inbred generation line	Second inbred generation data									Leaf width of broadest leaf				Height to tassel base (30 to 50 plants)				Average ear dimensions of second inbred generation, 40 to 50 plants			
	Ears examined	Ears in each class indicated					Diseased plants	Barren plants	Mode for number of kernel rows	First inbred generation, average for 20 plants at 50 days		Second inbred generation, average for 50 plants at maturity		First inbred generation		Second inbred generation		Total length per plant	Average ear length	Total diameter per plant	Average ear diameter
		Deeply creased dent	Creased dent	Dimpled dent	Shallow dimpled dent	Flint				Width	Proportion of check	Width	Proportion of check	Average height	Proportion of check	Average height	Proportion of check				
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		*Centimeters*	*Percent*	*Centimeters*	*Percent*	*Decimeters*	*Percent*	*Decimeters*	*Percent*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*
Castillear-1-2-1	24	37.5	41.7	8.3	12.5	.....	5.9	7.8	12	9.70	105.2	8.6	80.3	25.02	105.9	17.7	78.5	23.0	18.2	4.76	3.76
Castillear-1-3-1	24	.....	19.2	54.2	12.5	14.1	1.8	0	10–12	8.00	99.2	11.0	104.7	24.79	108.2	19.0	85.2	29.6	19.3	6.61	4.32
Castillear-1-3-2	45	4.4	33.4	46.7	13.3	2.2	1.8	3.6	10–12	8.00	99.2	9.1	86.5	24.79	108.2	17.8	81.3	23.7	19.7	5.10	4.22
Castillear-1-3-3	24	.....	12.5	8.3	45.9	33.3	0	13.5	12	8.00	99.2	8.3	81.5	24.79	108.2	13.7	62.1	16.1	12.8	4.75	3.80
Castillear-1-3-4	28	.....	25.0	42.9	25.0	7.1	0	15.7	.....	8.00	99.2	9.0	86.5	24.79	108.2	16.6	77.7	20.9	15.4	5.40	3.98
Castillear-1-4-1	35	2.8	17.2	25.7	42.9	11.4	0	17.6	12	8.88	110.8	10.1	95.0	24.10	101.4	16.8	79.8	26.2	15.6	6.84	4.06
Castillear-1-4-2	21	.....	47.7	28.6	14.3	9.4	0	4.3	.....	8.88	110.8	10.1	95.0	24.10	101.4	17.3	81.4	15.9	14.9	4.15	3.90
Castillear-1-4-3	31	3.2	64.6	22.8	9.4	.....	0	11.1	10–12	8.88	110.8	9.5	87.3	24.10	101.4	18.9	87.9	19.5	15.6	5.00	4.00
Castillear-1-5-1	40	2.5	75.1	17.4	5.0	.....	1.7	3.5	14	8.86	106.4	10.9	98.2	24.18	99.1	21.3	97.5	26.9	18.8	6.67	4.67
Castillear-1-5-2	37	.....	78.5	21.5	.....	.....	0	0	14	8.86	106.4	11.7	108.6	24.18	99.1	21.5	93.3	27.3	19.3	6.75	4.76
Castillear-1-1-1	45	2.2	53.3	31.2	13.3	.....	0	0	12–14	8.91	118.3	10.0	93.5	24.7	108.3	17.7	79.5	19.1	18.7	4.80	4.72
Vicens Flint-2-9-1	30	.....	10.0	50.0	23.4	16.6	1.8	0	10–12	7.52	98.8	9.2	80.5	22.21	103.7	17.7	78.1	20.4	16.2	5.16	4.12
Vicens Flint-2-9-2	21	.....	4.7	42.8	23.8	28.7	0	7.3	10–12	7.52	98.8	9.2	83.1	22.21	103.7	17.8	78.1	15.8	14.8	3.97	3.74
Vicens Flint-2-2-2	29	10.0	62.4	20.7	6.9	.....	0	19.3	10–12	8.73	106.8	9.2	85.2	25.14	111.8	19.7	89.5	19.9	17.4	5.22	4.55
Vicens Flint-2-3-1	24	.....	4.2	.....	29.2	66.6	2.78	6.3	10–12	7.95	97.0	7.7	71.0	22.56	97.7	14.9	65.1	15.2	14.8	4.34	4.01
Vicens Flint-2-4-2	28	.....	14.2	28.6	43.0	14.2	0	2.2	10	8.41	104.1	8.8	78.4	25.54	107.9	18.0	79.5	22.4	16.7	4.88	3.64
Vicens Flint-2-12-1	40	.....	12.5	37.5	30.0	20.0	0	0	10–12	8.67	106.3	9.6	87.2	22.90	100.8	17.0	76.7	16.9	16.3	4.35	3.98
Vicens Flint-2-14-2	18	5.0	11.1	27.8	27.8	27.8	11.8	8.8	12	9.33	106.6	7.3	67.3	23.20	100.0	16.8	77.0	16.4	14.2	4.87	4.22
Cacique-1-2-1	36	.....	52.8	33.3	13.9	.....	11.5	3.85	12–14	8.94	103.3	9.9	90.5	22.10	94.3	19.6	81.2	25.0	14.9	6.89	4.12
Cacique-1-2-4	24	.....	12.5	29.2	50.0	8.3	1.8	0	14	8.94	103.3	10.2	92.5	22.10	94.3	18.8	79.3	17.4	16.3	4.71	4.40
Cacique-1-50-1	18	.....	33.3	22.2	33.3	11.2	3.6	7.3	12	6.49	84.0	9.6	87.5	21.40	90.8	18.8	84.7	22.0	17.1	4.75	3.70
Cacique-1-50-2	9	11.1	44.5	33.3	11.1	.....	11.7	3.9	12	6.49	84.0	9.0	82.0	21.40	90.8	17.8	79.2	17.3	13.5	4.92	3.84
Cacique-1-67-1	22	4.5	50.0	31.8	13.7	.....	5.5	5.5	14	6.22	69.3	10.7	97.0	19.40	82.2	17.3	75.6	18.3	13.9	5.48	4.00
Cacique-1-78-2	41	.....	9.8	22.0	51.2	17.0	2.9	0	10–12	6.81	79.9	9.8	88.3	22.07	95.4	18.9	84.1	23.7	18.4	5.12	3.98
Cacique-1-93-1	25	.....	.....	8.0	32.0	60.0	6.6	3.3	12	6.65	67.7	8.6	79.7	17.90	80.7	13.5	63.2	19.1	14.0	4.96	3.64
Marcucci Droop-10-1-1	11	.....	88.9	11.1	.....	.....	0	10.5	12	8.02	93.9	9.9	88.3	21.60	105.9	21.0	92.9	23.3	22.1	4.83	3.58
Vicens-21-1-3	14	28.6	50.0	21.4	.....	.....	2.2	13.1	12	8.78	99.8	10.3	94.7	24.10	101.3	22.1	99.3	21.4	15.8	5.82	4.32
Yauco-Torre check	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	10.9	.....	.....	.....	22.2	.....	28.0	19.9	6.26	4.47

Relation Between Line-Variety Yield and Percentage of Soft Starch of the Inbred Pistillate Parent

The percentage of soft starch was determined by dissecting the soft starch from the halves of 50 kernels, 2 kernels being taken from near the center of each of 25 different ears of a given inbred line. Weights of dissected material were taken after the material had dried in an electric oven for 2 hours at 108° C. There was no marked relation between the yields of the line-variety hybrids and the proportion of soft starch in the inbred pistillate parent. The coefficient of correlation was low, only 0.198 ± 0.129. The 10 lines derived from parent ear Castillear-1 averaged 24.34 percent of soft starch and were generally superior in yield when outcrossed to the 7 lines derived from Cacique-1 which averaged 19.36 percent, and to the 7 lines from ear Vicens Flint-2 which averaged 17.34 percent.

Relation Between Line-Variety Yields and Mode for Degree of Denting

The progeny ears of each second-generation line were classified according to kernel types into five groups—deeply creased dent, creased dent, dimpled dent, shallow dimpled dent, and flint. Where fewer than 20 ears were classified no more were available due to barrenness, or disease, or weathering in the field. The correlation was positive ($r = 0.377 \pm 0.111$), but not high enough to justify a rigid elimination of flinty types of inbred lines.

Relation Between Line-Variety Yields and Percentage of Barren and Diseased Plants

The total percentage of barren and diseased plants of each inbred parent was negatively correlated with yield of the line varieties ($r = -0.441 \pm 0.025$). Of the 12 hybrids below average in yield, 9 had inbred parents with more than 10 percent of diseased and barren plants. Of the 7 highest yielding hybrids, 5 had inbred parents with less than 4 percent of diseased and barren plants, and only 1 had an inbred parent with as much as 10 percent.

Line-Variety Yields and Germination

The germination vigor is expressed as the foliage weight of 20 seedlings when 21 days old in percentage of that of Yauco-Torre seedlings grown in the same germination flat. The marked increase in this factor due to outcrossing with open-pollinated corn is shown in table 3. The inbred parents were, with two exceptions, inferior in seedling size to the Yauco-Torre corn, whereas the hybrids were generally superior, and on this basis the resulting generally superior yields could have been predicted. The average seedling weight superiority of hybrid over the open-pollinated staminate parent was 10.1 percent, and only 5 of 27 hybrids were inferior. The superior seedling vigor of most of the 27 hybrids is rather remarkable considering that the weight per 100 kernels of the Yauco-Torre pistillate parent was 37.5 g, whereas none of the hybrids exceeded 32.2 g in the xenia generation.

Considered within related groups, the line-variety hybrid with the most rapid early growth was the highest yielder; this was true in 4 groups of sib hybrids, not true in 1, and doubtful in 1 group. The correlation coefficient between the 26 line-variety yields and the

hybrid-seedling foliage weight was only 0.283 ± 0.124 and did not confirm the observations made on sib groups. This rather low correlation indicates that it is not safe to discard any but the very poorest germinators in the line-variety crosses.

Yields of the Line-Variety Hybrids in Relation to Leaf Width and Height of the Inbred Parent

Among the six groups of related line-variety hybrids the broadest leafed line within each group in the second inbred generation produced the highest yielding hybrid. There was one exception, that of the Castillear-1-4 derivatives. The correlation between leaf width of the inbred parent and line-variety yields ($r = 0.315 \pm 0.117$) was not so high as that for parent-progeny yields but tends to confirm observations made on groups of sib hybrids. There was no such relationship apparent between yield and height, there being equally as many low- as high-yielding sib hybrids with tall pistillate parents.

Line-Variety Yields and Yields of First Two Inbred Generations

A high and significant correlation was found between the line-variety yields and the average yields of the inbred parent in the first two inbred generations ($r = 0.638 \pm 0.0767$). Of the various characters studied yield is apparently the most dependable basis for elimination in inbred lines. All except 1 of the 9 lines that yielded 76 percent as much as the open-pollinated check variety in the first and second inbred generations, outyielded the check by 50 percent in line-variety combinations. Among 7 lines that were inferior to the check by 50 percent in the first 2 inbred generations, there were in the corresponding line-variety hybrids 3 low yielders, 2 high yielders, and 2 very high yielders. The elimination of all lines except those that averaged 76 percent of the check in the first 2 inbred generations would have retained 8 of 14 lines which produced very high yielding line-variety hybrids.

LINE-RECESSIVE YIELD TRIALS, MAYAGUEZ, 1928

Line-recessive yield trials were made at Mayaguez in 1928 to determine whether high-yielding hybrids could be produced by crossing lines inbred two or three generations and whether lines with larger plant growth and longer ears would produce higher yields when crossed with an unrelated, white line assumed as recessive because of white kernel color and inferiority in plant size, ear size, and yield. The spacing and size of plots were the same as those used in the 1927 line-variety yield trials. Growing conditions were good, and no abnormal weather interfered with grain production.

The material used in the 1928 yield trials consisted of hybrids between a white-kerneled line selfed three generations and 24 yellow-kerneled lines selfed two generations. The yellow lines were derived from six unrelated ears from corn districts near Aibonito, Barranquitas, Coamo, Lares, Morovis, and Peñuelas. With the exception of the ear from Peñuelas, the parent ears were selected on the basis of high total ear longisection area per plant and were entirely unrelated to the lines used in the 1927 line-variety yield trials.

The Peñuelas lines were derived from Castillear-1, the ear which was outstanding in the 1927 trials as a source of lines that gave high yield in line-variety combinations. The progeny of this ear was

open-pollinated during one season, and in the following generation 100 selfed lines were secured. From these 15 lines were retained which outgrew Yauco-Torre corn at 3 weeks. The first generation lines, nos. 21 and 50, were outstanding for healthy foliage and large plant growth in field trials, and from these the Peñuelas lines were derived.

The white line, Cacique-1-2-4-6 used as the staminate parent, makes a very inferior plant growth and produces small ears, 10–13 cm long and very small kernels. It yields between 8 and 10 bushels of shelled corn per acre, or about one fourth normal, and decidedly less than all the yellow-kerneled lines. Because of its decided inferiority, the white line was thought to afford a good test for the potential yielding values of the yellow lines, as only the more vigorous yellow types could be expected to give a high yield in F_1 combination. These hybrids were considered as contrasting with the line-variety hybrids in which the variety is the source of many dominant characters and an infinite variety of combinations is the result. In the former type of hybrid the yellow lines, being larger in ears, kernels, and plant growth than the staminate parent, constitute the principal source of dominant characters; the variety of combinations that result is not so great as in line-variety hybrids for it depends largely on the variability in the yellow lines.

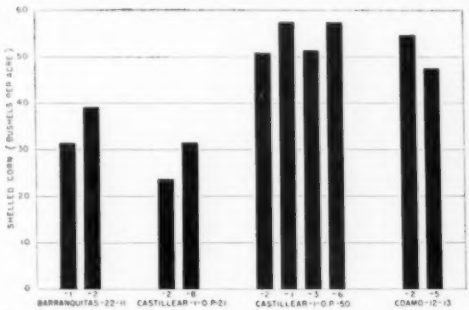

FIGURE 1.—Parallel performance in second-generation sibs, Mayaguez, 1928. The bars represent yields in bushels of shelled corn per acre of four groups of sib second-generation corn lines which have all been outcrossed with the same white line. Related hybrids were either all high yielding or all low yielding.

PARALLEL PERFORMANCE IN SECOND-GENERATION SIBS

Figure 1 shows diagrammatically the parallel performance of four different groups of sibs in hybrid combinations with the common white pollinator. The bars represent the average yields in bushels per acre. The bars representing yields of related hybrids are grouped together, and underneath in each case is given the first inbred generation line from which they were derived on the pistillate side. There was parallel performance in all four related groups; hybrids derived from Barranquitas-22-11 and Castillear-1-o.p.-21 were all low yielders while those from Coamo-12-13 and Castillear-1-o.p.-50 were all high yielders.

The superior crossing values of the four second-generation sib lines derived from Castillear-1-o.p.-50 are demonstrated when their hybrids are compared as a group with the Yauco-Torre corn. The eight plots outyielded the latter by an average of 15.51 bushels per acre. The odds are 1,732:1 that this difference is not entirely due to chance. Not only was the yield significantly greater but also the kernels had a brighter luster and the grain quality was superior, due to less decay at the tip ends of the ears. The parallel performance of these four groups of sibs when crossed with a nonrelated white recessive variety indicates that within the limited breeding material used it was feasible to select lines with high crossing values in the second inbred generation.

TABLE 5.—*Parent-progeny study of line-recessive [a] crosses and second-generation dominant parent lines, 1928*

Second-generation line parent	September 1927 data on second-generation lines						1928 data on line-recessive crosses											
							Average of 30 to 35 plants			Yield of air-dry shelled corn per acre						Yield of selection, average of plots K and L		Average height to tassel base (18 to 20 plants)	
										Plot K			Plot L						
	Average height to tassel base (30 plants)	Ears per 100 bearing plants	Total ear length per plant	Diameter of larger ears	Total longisection area [b]	Ears per 100 bearing plants	Total ear diameter	Total ear length	Total longisection area [b] of ears per plant	Selection	Average nearest 3 check [c] plots	Yield of selection as proportion of nearest 3 check plots	Selection	Check	Selection as proportion of check	Yield	Yield of selection as proportion of nearest 3 check plots	Hybrids	Yauco-Torre check
	Centimeters	*Number*	*Centimeters*	*Centimeters*	*Centimeters²*	*Number*	*Centimeters*	*Centimeters*	*Centimeters²*	*Bushels*	*Bushels*	*Percent*	*Bushels*	*Bushels*	*Percent*	*Bushels*	*Percent*	*Decimeters*	*Decimeters*
Aibonito-4-5-3	121.9	133	16.8	3.14	52.1	114	4.96	18.07	89.6	35.3	34.4	102.7	33.7	44.83	75.2	34.5	88.6	18.2	19.2
Aibonito-4-8-4	136.1	143	16.8	3.53	59.4	106	4.74	17.26	81.9	38.4	34.4	111.6	41.2	44.83	92.1	39.8	101.8	19.1	19.5
Aibonito-4-12-1	160.9	174	23.4	3.50	81.9	109	5.06	19.36	98.0	45.4	37.6	120.8	39.1	43.30	90.2	42.25	105.5	19.3	20.1
Aibonito-4-15-1	128.1	162	19.7	3.55	70.0	111	4.83	18.32	88.5	34.8	34.7	100.2	42.1	37.20	113.1	38.45	106.6	18.4	20.5
Barranquitas-22-9-1	136.4	160	23.2	3.31	76.7	123	5.29	20.63	109.2	21.2	34.05	62.3	20.6	33.40	61.7	20.9	62.0	17.9	20.0
Barranquitas-22-11-1	177.4	156	20.8	2.76	57.4	130	5.66	20.54	116.1	20.4	33.43	61.1	40.6	34.4	118.0	30.5	89.5	19.4	18.3
Barranquitas-22-11-2	185.6	100	13.0	3.40	44.2	122	5.47	20.02	109.6	34.5	31.70	108.8	44.0	36.33	121.0	39.25	114.9	19.9	18.7
Barranquitas-22-15-1	141.7	169	23.8	2.77	66.0	124	5.40	21.40	115.6	34.6	33.40	103.4	39.3	36.90	106.4	36.95	104.9	19.8	19.7
Coamo-12-1-1	121.9	109	17.1	3.36	57.6	100	4.54	17.81	81.2	38.3	41.10	93.1	34.9	33.53	104.2	36.6	98.6	17.2	19.3
Coamo-12-4-3	126.7	174	23.2	3.05	70.7	123	6.02	20.80	125.0	56.8	46.43	122.5	39.2	37.30	105.2	48.1	113.8	18.2	18.1
Coamo-12-13-2	141.8	170	21.9	3.47	76.0	121	5.32	20.85	110.9	53.8	47.90	112.2	53.9	38.97	138.4	53.85	125.3	19.4	19.5
Coamo-12-13-5	120.9	165	20.2	3.09	62.5	133	5.91	20.77	122.7	51.0	47.90	106.4	42.3	38.9	108.7	46.65	107.5	17.6	19.0
Lares-6-4-2	142.5	138	17.6	3.05	53.7	109	4.65	20.07	93.3	42.3	46.50	91.0	33.2	39.63	83.7	37.75	87.3	18.1	19.5
Lares-6-7-2	168.4	166	21.8	3.56	77.6	117	5.37	19.76	106.2	47.3	46.50	101.7	36.0	39.63	90.8	41.65	96.2	21.0	19.8
Morovis-6-2-3	139.8	135	20.5	2.59	53.1	103	4.91	19.95	98.0	30.2	48.70	62.0	50.5	38.13	132.3	40.35	97.1	17.5	19.6
Morovis-6-6-1	141.0	169	21.0	4.11	86.3	139	7.54	25.15	189.8	70.5	53.40	132.0	58.3	39.1	149.1	64.4	140.5	18.5	20.4
Morovis-6-7-4	126.7	142	14.5	3.12	45.2	116	5.35	20.23	108.2	39.9	52.63	75.7	39.2	39.9	98.2	39.55	86.9	17.7	20.6
Castillear-1-3-o.p.-7-2	116.1	145	17.1	3.04	51.8	115	4.69	17.42	81.9	32.9	38.6	85.3	29.0	40.8	71.0	30.95	78.1	16.2	18.1
Castillear-1-o.p.-21-2	96.1	132	15.5	3.58	55.5	103	4.83	18.53	89.5	20.6	45.73	45.1	25.1	40.6	61.7	22.85	53.4	17.2	18.2
Castillear-1-o.p.-21-8	116.6	139	17.0	4.14	70.4	123	5.65	16.64	94.0	24.9	48.80	51.0	38.0	40.63	93.4	31.45	72.2	16.4	19.0
Castillear-1-o.p.-50-1	158.1	147	23.0	3.87	89.0	133	6.06	21.76	132.0	63.5	44.77	142.0	51.2	40.73	125.7	57.35	133.8	18.8	20.2
Castillear-1-o.p.-50-2	174.5	168	20.6	3.69	76.0	113	5.09	19.47	99.1	61.9	40.13	154.1	38.3	36.27	105.8	50.1	129.9	19.6	20.3
Castillear-1-o.p.-50-3	153.2	164	21.2	3.69	78.3	144	6.23	23.23	144.8	62.3	36.06	173.0	40.3	34.60	116.6	51.3	144.8	20.7	19.8
Castillear-1-o.p.-50-6	164.2	182	21.5	3.80	81.9	151	6.19	23.43	144.8	56.8	37.93	149.8	57.0	36.70	155.0	56.9	152.4	20.2	19.2

[a] Common pollinator = Castillear-1-2-4-6, a white recessive, inbred 3 times, which produces 10 bushels of shelled corn per acre, has a total ear length per plant of 13 centimeters, and a height of 11.1 decimeters to tassel base.

[b] Diameters of larger ears only were used to calculate total longisection area per plant, as incomplete pollination prevented the determination of the diameters of the other ears of the inbred parents.

[c] Yauco-Torre = corn check variety.

As may be observed in the data on height in table 5 the growth of the check plants which grew in rows adjoining the hybrids was not markedly affected by the plant size of the latter. As a rule, the check grew just as tall next to the taller hybrids as it did in the rest of the field. The competition between varieties does not appear to have been marked, and there were several instances of high yields of hybrids which were quite inferior to the check in height, i.e., those of Morovis-6-6-1 and Coamo-12-13-2.

PARENT-PROGENY CORRELATION STUDIES, 1928 CROP

Since the white staminate parent was almost homozygous for kernel size, color, ear length, and height, it was predicted that most of the variability in the hybrids would be due to the influence of the yellow parents. It was therefore thought that high correlations in plant height and yield would be secured between the line-recessive crosses and inbred yellow parents. Unfortunately poor pollination due to violent storms in 1927 prevented the securing of data on yield of parent lines. There was, however, a high correlation for longisection area of ears per plant between line-recessive hybrids and the respective yellow-line parents ($r=0.549\pm0.096$), and as was predicted, the parent-progeny correlation for height was high ($r=0.834\pm0.043$). Since hybrid yields were also strongly correlated with these characters of the inbred parents, $r=0.595\pm0.089$ for yield and height and $r=0.540\pm0.097$ for yield and total ear longisection area per plant, it follows that they could have been used as a basis for elimination in the second inbred generation. These results tend to corroborate those secured by Jenkins [9] on lines inbred 3 or 4 generations.

The correlations between line-recessive hybrid yields and ear length or ear diameter of inbred parents were nearly double those for 1927 line-variety yields and the same characters of the inbred parents, as shown below. Apparently much less variability in hybrid yield and hybrid plant growth resulted from the use of the open-pollinated variety than through the use of the recessive line. As a test for relative dominance among inbred lines the line-recessive method appears to be more effective. In the line-variety hybrids the dominant characters contributed by the open-pollinated variety tended to obscure those of the selfed lines.

Characters correlated with line-variety yields:	*Coefficients of correlation*
Average yield of first and second inbred generations	0.638±0.0767
Seedling foliage weight of hybrids at 3 weeks	.283± .124
Mode for degree of denting of inbred parent	.377± .111
Percentage of soft starch of inbred parent	.198± .129
Total ear length per plant of inbred parent	.189± .125
Total ear diameter per plant of inbred parent	.175± .125
Leaf width of inbred parent	.315± .117
Percent of diseased and barren plants of inbred parent	−.441± .025
Characters of dominant inbred parents correlated with line-recessive yields:	
Height to tassel base	.549± .095
Total ear longisection area per plant	.540± .097
Total ear length per plant	.384± .117
Total ear diameter per plant	.386± .117
Parent-progeny characters correlated:	
Total longisection area of ears per plant of line-recessive hybrids and the same of dominant yellow inbred parents	.549± .096
Height to tassel base of line-recessive hybrids and the same of dominant inbred parents	.834± .043

[9] JENKINS, M. T. See footnote 7.

The average yield increase over Yauco-Torre check corn was nearly 10 times as great for the 26 line varieties as for the 24 line-recessive hybrids. The average inferiority of the line recessives is apparently owing to the high proportion of lines which were not outstanding for dominant characters and were inferior for plant growth. The yields of the more promising of the line-recessive hybrids, however, compared quite well with those of the high-yielding line varieties.

UTUADO LINE-VARIETY YIELD TRIALS [10]

Line-variety yield trials were made at Utuado to compare yields of line-variety hybrids with those of their pistillate variety parents and with the corn produced on the farm of Tomás García.

Three line-variety hybrids produced at the Isabela substation and five produced through the cooperation of Central Coloso near Isabela were compared with corn locally produced on the García farm and with the pistillate parent varieties. The staminate parents were Castillear-1-5-1 composite and Castillear-1-5-2 composite, derived from the same first generation line; after 4 to 5 generations of self-pollination 6 and 8 sibs, respectively, were lumped. The parent lines of both staminate parents had given high yields in line-variety crosses in 1927. The pistillate parents consisted of corn varieties collected in various parts of the island. In each instance corn was chosen from farms where the practice had been for seed corn to be saved from year to year. The objective was to find distinct types of corn. With the exception of the Aguadilla corn, however, which had dark-orange seed color, red cobs, and was fairly uniform for dimpled to smooth dent kernels and long tapering ears, all the other varieties were similar in type, i.e., a smooth dent with ears of medium length varying in kernel color from light yellow to orange. The corn produced on the García farm came originally from Yauco and was very similar to the Yauco-Torre corn.

The corn from the García farm was used as a check and was planted in every third plot. Hills were spaced 3 feet apart each way, and the corn was thinned to two plants per hill. The plots were 2 rows wide and 208 feet long. Three plots, one thirty-fifth of an acre in area, were planted to each hybrid. The soil was a loose sandy loam, typical of the cornland of the Utuado district. The field was well drained and not under irrigation. A heavy rain that fell a few days after planting washed out the seeds and made it necessary to replant the entire field. Owing in part to adverse conditions at the start, and in part to a steep somewhat stony slope at one end of the field, yields were very erratic.

[10] Cooperation with the Insular Department of Agriculture.

TABLE 6.—*Yields*[a] *per acre of air-dry shelled corn in the Utuado line-variety-hybrid trials, the yields of hybrids being compared with those of pistillate parents*

Plot no.	Hybrid	Yield of hybrids	Yields in adjoining plots of pistillate parents	Increase or decrease	Deviation from mean, $D-M$ [b]	$(D-M)^2$ [b]
		Bushels	*Bushels*	*Bushels*	*Bushels*	*Bushels*
9	Juarbe-1	29.1	27.7	+1.4	−1.53	2.341
30	Juarbe-1	17.8	21.5	−3.7	−6.63	43.957
51	Juarbe-1	13.1	15.2	−2.1	−5.03	25.301
12	San Sebastián-1	26.1	22.9	+3.2	+.27	.073
33	San Sebastián-1	22.2	20.1	+2.1	−.83	.689
54	San Sebastián-1	12.9	13.2	−.3	−3.23	10.433
15	Peñuelas-1	26.8	17.6	+9.2	+6.27	39.313
36	Peñuelas-1	24.6	17.5	+7.1	+4.17	17.389
18	Coamo-1	25.7	20.5	+5.2	+2.27	5.153
60	Coamo-1	14.2	15.7	−1.5	−4.43	19.625
21	Barranquitas-1	24.0	15.3	+8.7	+5.77	33.293
42	Barranquitas-1	12.7	11.4	+1.3	−1.63	2.657
63	Barranquitas-1	16.8	9.3	+7.5	+4.57	20.885
	Mean			2.93		

[a] $N=13$; $S.D.=4.123$; $Z=0.71$; Odds=654:1.

[b] In making these calculations the differences between the pistillate parents were disregarded, and the yields of the 13 hybrid plots were treated as though they were repetitions of the same cross. Each hybrid plot adjoined a plot of its pistillate parent making direct comparisons fair. All hybrids listed here had the same staminate parent, Castillear-1-5-1 composite.

TABLE 7.—*Yields per acre of air-dry shelled corn in the variety trial, Utuado, P.R.*[a]

Variety	Plot no.	Yield based on field weight	Interpolated yields of checks	Plot no.	Yield based on field weight	Interpolated yields of checks	Plot no.	Yield based on field weight	Interpolated yields of checks	Average increase or decrease as compared with check (3 plots)	Odds indicating significance of difference
		Bushels	*Bushels*		*Bushels*	*Bushels*		*Bushels*	*Bushels*	*Bushels*	
Garcia	1	19.7									
Aguadilla-2	2	31.8	21.00	23	18.6	23.76	44	14.5	10.53	+3.203	2.53:1
Peñuelas-2	3	28.8	22.30	24	23.8	20.23	45	9.6	12.16	+2.50	3.45:1
Garcia	4	23.6		25	16.7		46	13.8			
Juarbe-2	5	35.8	23.60	26	22.4	16.83	47	13.3	12.34	+6.270	9.16:1
Aguadilla-1	6	26.6	23.60	27	20.9	16.96	48	15.5	11.07	+3.79	166:1
Garcia	7	23.6		28	17.1		49	9.7			
Juarbe	8	27.7	22.64	29	21.5	16.60	50	15.2	11.77	+4.463	262:1
Juarbe-1	9	29.1	21.67	30	17.8	16.10	51	13.1	13.83	+2.80	4.4:1
Garcia	10	20.7		31	15.6		52	15.9			
San Sebastián	11	22.9	20.30	32	20.1	17.10	53	13.2	15.04	+1.253	3.28:1
San Sebastián-1	12	26.1	19.90	33	22.2	18.60	54	12.9	14.17	+2.843	4.96:1
Garcia	13	19.5		34	20.1		55	13.3			
Peñuelas	14	17.6	19.80	35	17.5	19.16	56	11.4	13.0	−1.82	67.3:1
Peñuelas-1	15	26.8	20.10	36	24.6	18.23	57	(b)		+6.535	377:1
Garcia	16	20.4		37	17.3		58	(b)			
Coamo	17	20.5	21.13	38	15.7	15.73	59	15.7	12.1	+.98	1.9:1
Coamo-1	18	25.7	21.86	39	(b)		60	14.2	11.8	+3.12	8.03:1
Garcia	19	22.6		40	12.6		61	11.5			
Barranquitas	20	15.3	24.17	41	11.4	11.36	62	9.3	12.2	−3.91	6.42:1
Barranquitas-1	21	24.0	25.74	42	12.7	10.13	63	16.8	12.9	+1.577	4.26:1
Garcia	22	27.3		43	8.9		64	13.6			

[a] Hybrids have number suffixed to name of pistillate parent. Plot size=one thirty-fifth of an acre consisting of 2 rows, 3 feet apart and 208 feet long. Hills were spaced 3 by 3 feet. Corn plants were thinned down to 2 plants per hill. All plots were parallel to each other and fronted on the same side of the field.

[b] Omitted on account of incomplete harvest.

No significant differences were found between the yields of individual line-variety hybrids and their respective pistillate parents which grew in each case in adjoining plots (table 6). It is probable that a larger number of replications would have demonstrated a

superiority for some of the individual hybrids as, taken as a group, the 13 plots of hybrids pollinated by Castillear-1-5-1 averaged 2.93 bushels per acre more than the pistillate parents. The 13 hybrid plots were treated as though they were repetitions of the same cross. The odds were 654:1 that this difference was not due to chance. The weights of air-dry shelled corn were based on a shrinkage sample of 100 ears collected, half from corn grown on the García farm and half the interplanted varieties; the moisture content was assumed to be the same for all varieties, as differences in type of corn or degree of maturity were not marked.

TABLE 8.—*Yields[a] per acre of air-dry shelled corn in the Utuado line-variety trials the yields of hybrids being compared with those of the García check corn*

[Common pollinator or staminate parent=Castillear-1-5-1 composite, harvested July 28, 1931]

Hybrid	Yield of hybrids	Interpolated yields of García check corn	Increase or decrease	Deviation from mean, $D-M$ [b]	$(D-M)^2$ [b]
	Bushels	*Bushels*	*Bushels*	*Bushels*	*Bushels*
Juarbe-1	29.1	21.67	+7.43	+4.159	17.297
	17.8	16.10	+1.70	−1.571	2.468
Peñuelas-1	13.1	13.83	−.73	−4.001	16.008
	26.8	20.10	+6.70	+3.429	11.758
San Sebastián-1	24.6	18.23	+6.37	+3.099	9.604
	26.1	19.90	+6.20	+2.929	8.579
Coamo-1	22.2	18.6	+3.60	+.329	.108
	12.9	14.17	−1.27	−4.541	20.621
Barranquitas-1	25.7	21.86	+3.84	+.569	.324
	14.2	11.80	+2.40	−.871	.759
Aguadilla-1	24.0	25.74	−1.74	−5.011	25.110
	12.7	10.13	+2.57	−.701	.491
	16.8	12.90	+3.90	+.629	.396
	26.6	23.60	+3.00	−.271	.073
	20.9	16.96	+3.94	+.669	.448
	15.5	11.07	+4.43	+1.159	1.343
Mean			+3.271		

[a] $N=16$; $S.D.=2.686$; $Z=1.22$; Odds=4,991:1.
[b] In making these calculations the differences between the pistillate parents were disregarded, and yields of the 16 hybrid plots were treated as though they were repetitions of the same cross.

TABLE 9.—*Yields[a] per acre of air-dry shelled corn in the Utuado line-variety trials, the yields of hybrids being compared with those of the García check corn*

[Common pollinator or staminate parent=Castillear-1-5-2 composite]

Hybrid	Yield of hybrids	Interpolated yields of García corn check	Increase or decrease	Deviation from mean, $D-M$ [b]	$(D-M)^2$ [b]
	Bushels	*Bushels*	*Bushels*	*Bushels*	*Bushels*
Aguadilla-2	31.8	21.0	+10.8	+6.82	46.512
	18.6	23.76	−5.16	−9.14	83.540
Peñuelas-2	14.5	10.53	+3.97	−.01	.000
	28.8	22.3	+6.50	+2.52	6.350
Juarbe-2	23.8	20.23	+3.57	−.41	.168
	9.6	12.16	−2.56	−6.54	42.772
	35.8	23.6	+12.20	+8.22	67.568
	22.4	16.83	+5.57	+1.59	2.528
	13.3	12.34	+.96	−3.02	9.120
Mean			+3.98		

[a] $N=9$; $S.D.=5.363$; $Z=-0.74$; odds=27:1.
[b] In making the calculations, differences between the pistillate parent varieties from Aguadilla, Peñuelas, and the Juarbe farm were disregarded, and the 9 plot yields were treated as though they were repetitions of the same cross.

As shown in table 7 there were significant differences between the García check corn and two of the hybrids, Peñuelas-1 and Aguadilla-1. The former averaged 3.79 bushels more of the shelled corn per acre than the check and the latter was superior by 6.52 bushels. In the case of the Castillear-1-5-1 hybrids the results are more conclusive if a general comparison is made between the two groups of hybrids and the García corn. The 16 plots of hybrids pollinated by Castillear-1-5-1 composite averaged 3.271 bushels per acre more than the check corn (table 8). The odds are 4,999:1 that this difference is not due to chance. The nine plots of hybrids pollinated by Castillear-1-5-2 composite averaged 3.98 bushels per acre more than the check (table 9). The odds are 27.1 that this difference is not due to chance.

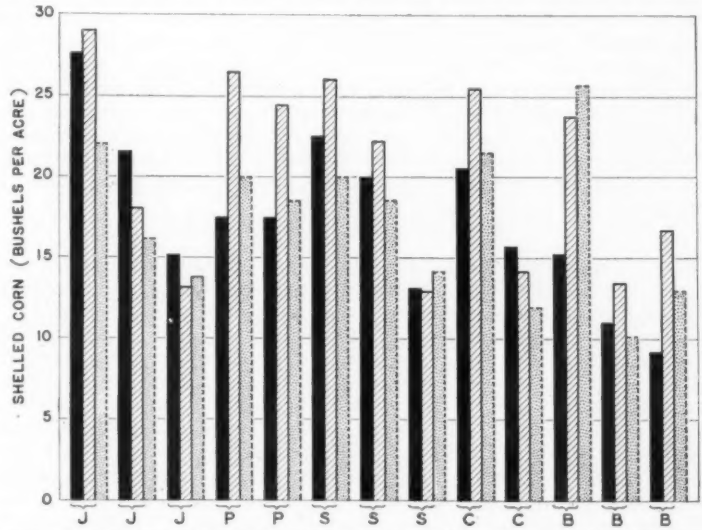

FIGURE 2.—Yields of line-variety hybrids and varieties at Utuado, April 1931. The black bars represent yields of varieties, the hatched bars those of the line-variety hybrids, and the dotted bars those of the García check corn. J=Juarbe, P=Peñuelas, S=San Sebastián, C=Coamo, and B=Barranquitas.

The general superiority of the Castillear-1-5-1 line-variety hybrids is shown graphically in figure 2, and the places of origin of the open-pollinated varieties is indicated in the legend. Each group of bars represents the yields of three adjoining $\frac{1}{35}$-acre plots of a variety, and that of a cross between that variety and the inbred line, Castillear-1-5-1, and that of García check corn. The Juarbe pistillate parent was superior to its hybrid with Castillear-1-5-1 and compared well with all of the hybrids in grain production.

DISCUSSION OF RESULTS

The general superiority of the yields of the 27 line-variety crosses to those of the variety parent indicated that this use of second-generation lines would prove effective in increasing yields of corn grown near Yauco.

There were, among both 1927 and 1928 crosses, 10 sib groups, 9 of which gave a parallel performance for yield. This affords some

evidence that the parent lines inbred only two generations were approaching uniformity for characters that make for yield in cross-bred combination.

A high parent-progeny correlation for yield was secured in 1927, and a still higher one for hybrid yield and total longisection area of ears of inbred parent was secured in 1928. It seems that within the material studied the second-generation lines with the highest crossing values could have been selected prior to crossing on the basis of yield or characters directly associated with yield.

Complete restoration of hybrid vigor resulted from crossing the second-generation lines with a nonrelated open-pollinated variety. Difference in degree of expression of a character contributed by inbred lines tended therefore to be obscured in line-variety combination. Among line recessives there were very high parent-progeny correlations for both height and ear longisection area, and the variability in the germ plasm contributed by the more vigorous yellow-kerneled parents was not apparently affected by the contribution of the so-called recessive parent. Presumably the prepotency of second-generation lines as regards a particular character could be studied to better advantage if they were outcrossed with a line known to be recessive for that character than if crossed with an open-pollinated variety.

SUMMARY AND CONCLUSIONS

Ears were selected from Lajas, Peñuelas, and Jayuya in 1923 and tested for seedling vigor. The ears which were superior in seedling size and drought resistance were placed in ear-to-row yield trials.

Lines were inbred two generations from the three highest yielding parent ears from the three districts and compared in yield and plant characters. They were then crossed with Yauco-Torre, an unrelated open-pollinated variety. In 1927 yield trials were conducted at Mayaguez. The 27 line-variety hybrids taken as a group were significantly superior in yield to the Yauco-Torre corn. Castillear-1-5-1 × Yauco-Torre was significantly superior to the other hybrids.

In 5 of 6 groups of sib line-variety hybrids a parallel performance was observed; they were either all high yielding or low yielding.

Correlations were determined between line-variety yields and various characters of inbred parents. The correlation was significant and positive for yield and average yield of first and second inbred generations. The correlation was negative and fairly high, but not significant, for hybrid yield and percentage of barren and diseased plants. Correlation of hybrid yield with mode for degree of denting of kernel, and leaf width of inbred parent were positive and fairly high but not significant. Correlations of hybrid yield with inbred parent characters, percentage of soft starch, and seedling foliage weight were positive but very low. These correlations indicated that within the material studied the average yield of the first and second inbred generations was the most dependable basis for elimination.

Five parent ears from five different districts were selected for high total longisection area of ears per plant and self-pollinated. In 1928 yield trials were conducted at Mayaguez comparing hybrids between second inbred generation derivatives of these ears and a line, assumed as recessive, which had been selfed three times. The line-recessive

hybrid yields were not as superior to the Yauco-Torre check corn as were those of the line-variety hybrids. Parallel performance was noted among hybrids secured from four groups of second-generation sibs. Significant correlations were secured between line-recessive hybrid yields, and plant height and total longisection area of ears per plant of the yellow-kerneled inbred lines. Both of these characters of the yellow-kerneled lines appeared dependable as a basis for elimination in the second inbred generation.

Yields significantly superior to Yauco-Torre corn were secured from four sib lines derived from Castillear-1-o.p.-50 by outcrossing them in the second inbred generation with a third-generation line.

In 1931 line-variety crosses between the composites, Castillear-1-5-1 or Castillear-1-5-2 and a number of native varieties outyielded the corn grown on the García farm in the Utuado district. These lines gave superior yields in the second inbred generation outcrossed with the open-pollinated native field corn and continued to do so after the fourth to fifth inbred generation. The margin of superiority does not appear to have been increased by additional generations of inbreeding prior to crossing.

HYPERPARASITISM IN THE CASE OF SOME INTRODUCED LEPIDOPTEROUS TREE DEFOLIATORS[1]

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INTRODUCTION

It has long been known that hyperparasites have a considerable effect on the abundance of primary parasites. In recent years, as more interest has been centered upon the biological control of injurious insects, the problem of the interrelations of parasites, not only between the various primary parasites themselves but also between the primary parasites and the hyperparasites, has called for particular study. It has been considered that one of the chief causes of the reduction in numbers of the parasites imported into New England to aid in the control of the gypsy moth, the brown-tail moth, and other moths has been the activities of hyperparasites. In this paper it is proposed to make available the present information on (1) the extent to which these imported parasites are attacked by secondary parasites of the lepidopterous hosts, (2) the species concerned, and (3) the relative importance of each.

To find out the extent of hyperparasitism in the case of the gypsy moth (*Porthetria dispar* L.), the brown-tail moth (*Nygmia phaeorrhoea* Don.), the satin moth (*Stilpnotia salicis* L.), and the oriental moth (*Cnidocampa flavescens* Walk.) collections of cocoons or puparia of the parasites of these moths were made at various points in their respective infested areas of New England during a period of 4 years, from 1929 to 1932, inclusive.

There was some difficulty in obtaining collections of most of the species of primary parasites in the field. It was thought desirable to make collections at the same localities over a series of years, and as a result many collections were small, especially where the host population was small during the entire period.

Special efforts were made to ascertain the maximum hyperparasitization by collecting cocoons or puparia as near the time of issuance of the primary parasite as possible. In many cases some of the primary parasites had already issued, and these were taken into account with the others. No matter when the collections were made, there were a few cocoons or puparia that had been subjected to the attack of

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secondaries for only a short time, and the inclusion of these altered the percentages to a certain extent; but as special precautions were taken and, in most cases, good-sized samples obtained, it is believed that a fair picture is presented of the nature and extent of parasitization of these primary parasites under average field conditions.

In the discussion of the primary parasites the reader is referred in each case to the paper that treats the particular species in most detail. Where no reference to literature cited is given, the information is in the form of unpublished notes on file at the Bureau of Entomology laboratory at Melrose Highlands, Mass.

Unless otherwise noted, the dead immature forms of hyperparasites that could not be positively identified, but that were encountered during the examination of the cocoons or puparia that failed to yield adults, are classed in the tables as "undetermined species."

Except for *Sturmia scutellata* R. D., all cocoons and puparia collected for this study were isolated in small glass vials stoppered with cotton plugs, which permitted accurate determinations of the percentages killed by hyperparasites.

PARASITIZATION OF PRIMARY PARASITES

HYMENOPTERA, BRACONIDAE

APANTELES LACTEICOLOR VIERECK

Apanteles lacteicolor (*5, pp. 194–201*),[3] a larval parasite of the brown-tail moth, forms its cocoons in the hibernation webs of its host about 10 days after the host larvae have begun to feed in the spring. The cocoon is very delicate and is easily crushed in handling, which may account in part for the large number of cocoons from which neither *Apanteles* nor hyperparasites were reared. Most of the cocoons of *A. lacteicolor* are formed so far in the interior of the web that to attack them the adult hyperparasites not already within it must enter and search through a labyrinth of passages for them. The extreme discomfort experienced while working with the brown-tail moth because of the irritating barbed and brittle hairs of the caterpillars has been a deterrent to the accumulation of any very large collections of its parasites. Consequently conclusions which may appear to be based on rather small numbers of cocoons or puparia are in reality founded on collections requiring a great deal of perseverance in their proper handling, and the small numbers must be considered as unavoidable under the circumstances.

In 1929 four collections totaling 457 cocoons of *Apanteles lacteicolor* were made in four localities in the New England area infested with the brown-tail moth; and in 1930 six collections totaling 1,067 cocoons were made in six localities, two in the same places as in the previous year. The percentage known to have been killed by hyperparasites of the brown-tail moth in individual collections ranged from 0 to 22, and the average for all collections in both years was 5 percent. In table 1 it is shown that 22, or 1.5 percent, of the cocoons of *A. lacteicolor* were found to have produced adults of *Eupteromalus nidulans*. Although a small percentage of the total, it represents nearly half of the number killed by hyperparasites the species of which could be determined. Yet this degree of parasitization is not

[3] Reference is made by number (italic) to Literature Cited, p. 376.

surprising, for *Eupteromalus* finds these cocoons immediately available as it attains maturity following its hibernation as a primary parasite within the webs of the brown-tail moth. In fact, considering the large number of webs that were examined, and that the majority of these webs contained from a few to a hundred or more hibernating *Eupteromalus* per web, it is surprising that no greater number of *A. lacteicolor* were attacked. It is interesting that *Eupteromalus* acts both as a primary parasite of the brown-tail moth and as a secondary parasite through *A. lacteicolor* in the same web. In a previous publication (*9, p. 53*) the writer showed that *Eupteromalus* is far more important as a primary parasite under field conditions, especially when attacking the satin moth.

TABLE 1.—*Parasitization of Apanteles lacteicolor, 1929–32*

Species of parasite	Cocoons of *A. lacteicolor*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Eupteromalus nidulans (Thomson)	22	1.5	22	22	1.0
Monodontomerus aereus Walker	13	.9	13	13	1.0
Hemiteles tenellus (Say)	4	.3	4	4	1.0
Gelis bucculatricis (Ashmead)	2	.1	2	2	1.0
Hypopteromalus inimicus Muesebeck	2	.1	2	2	1.0
Dibrachys boucheanus (Ratzeburg)	2	.1	2	3	1.5
Eurytoma appendigaster (Swederus)	2	.1	2	2	1.0
Pleurotropis tarsalis (Ashmead) *	1	.1	1	1	1.0
Undetermined species	28	1.8	28		
Total killed by parasites	76	5.0			
Adults of *A. lacteicolor* issued	840	55.1			
Dead from unknown causes	608	39.9			
Total	1,524	100.0			

* Probably a secondary to *A. lacteicolor*.

Monodontomerus aereus also spends the winter within the brown-tail-moth hibernacula, but as adults and not in such numbers as does *Eupteromalus*. Most of the individuals of *M. aereus* overwinter in the cocoon masses of the brown-tail moth, as mentioned by Muesebeck (*7, p. 447*), who adds (*p. 457*) that this species is a negligible factor in the parasitization of *A. lacteicolor*. The present figures bear out that statement.

The two most important species of secondary parasites are the ones that pass the winter in the brown-tail-moth hibernacula. This adds weight to the theory that hyperparasitism depends to a large degree on the extent of exposure.

The 5 percent mortality of *Apanteles lacteicolor* shown to be due to parasitization is certainly not all that might be charged to this, since it is at present impossible to determine with accuracy the number of primary parasites that are killed by hyperparasites when no eggs are laid or when they fail to develop. Many are killed by the hyperparasites in puncturing their primary hosts for purposes of oviposition or of feeding. For example, the writer found (*9, p. 43*) that in laboratory experiments *Eupteromalus nidulans*, acting as a primary parasite of the satin moth, laid eggs upon only one third of the total number of host larvae that it killed. Muesebeck and Dohanian (*8, pp. 6–7*)

likewise show that such feeding is very common among most of the species of hyperparasites concerned in this study. It is probable that a very large number of primary parasites are killed in this way. This notation applies to all the species discussed in this paper, although generally not to such an extent as with *A. lacteicolor*, 40 percent of the cocoons of which produced neither *Apanteles* nor hyperparasites.

Apanteles melanoscelus (Ratzeburg)

Muesebeck and Dohanian (*8*) studied the parasites of *Apanteles melanoscelus* (*2*), a valuable larval parasite of the gypsy moth, in considerable detail. Therefore, to avoid duplication, no special effort was made to secure a large collection of cocoons, but it was thought desirable to obtain a number sufficient for comparing the incidence of the various species of hyperparasites with their abundance when attacking other hosts during the same seasons and if possible in the same areas. In addition, the data in table 2 are presented in such a way as to show the comparative abundance of the different species when attacking each of the two generations of *A. melanoscelus*.

Table 2.—*Parasitization of Apanteles melanoscelus, 1929–30*

FIRST GENERATION

Species of parasite	Cocoons of *A. melanoscelus*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Eurytoma appendigaster (Swederus)	56	13.8	56	56	1.0
Hemiteles tenellus (Say)	36	8.9	36	36	1.0
Gelis bucculatricis (Ashmead)	15	3.7	15	15	1.0
Dibrachys boucheanus (Ratzeburg)	9	2.2	9	23	2.6
Mesochorus vitreus Walsh	2	.5	2	2	1.0
Gelis urbanus (Brues)	1	.2	1	1	1.0
Dimmockia incongruus (Ashmead)	1	.2	1	4	4.0
Undetermined species	12	3.0			
Total killed by parasites	132	32.5			
Adults of *A. melanoscelus* issued	210	51.7			
Dead from unknown causes	64	15.8			
Total	406	100.0			

SECOND GENERATION

Eurytoma appendigaster (Swederus)	173	69.2	[a] 156	156	1.0
Dibrachys boucheanus (Ratzeburg)	13	5.2	13	59	4.5
Gelis bucculatricis (Ashmead)	4	1.6	4	4	1.0
Eupelminus saltator (Lindeman)	1	.4	1	1	1.0
Gelis inutilis Cushman	1	.4	1	1	1.0
Habrocytus phycidis Ashmead	1	.4	1	1	1.0
Hypopteromalus inimicus Muesebeck	1	.4	1	1	1.0
Pleurotropis nawai (Ashmead)	1	.4	1	1	1.0
Undetermined species	16	6.4			
Total killed by parasites	211	84.4			
Adults of *A. melanoscelus* issued	25	10.0			
Dead of unknown causes	14	5.6			
Total	250	100.0			

[a] Total number of cocoons giving secondary parasite adults.

In their table 1 (*8*, *p. 10*), Muesebeck and Dohanian showed that the first-generation cocoons of *Apanteles melanoscelus* were heavily parasitized, only 28.4 percent producing adults of *Apanteles*, whereas

52.8 percent produced hyperparasites and 18.8 percent yielded neither. These figures were based on nine collections totaling 2,164 cocoons. In the present study only 406 first-generation cocoons were collected, but they were obtained from 19 localities and during a 3-year period.

It will be seen that the total parasitization of the first generation of *Apanteles melanoscelus* here reported is 20 percent less than that found by Muesebeck and Dohanian. It is doubtful whether this difference can be attributed to periodic variation. Of course the present percentages are based on a much smaller number of cocoons collected, and thus may be less representative of the area as a whole, but on the other hand the cocoons taken by Muesebeck and Dohanian were probably obtained only in fairly heavy infestations of both the gypsy moth and its primary parasite *A. melanoscelus*, and therefore may not be as fair a sample of average conditions as is the smaller number reported here. Whatever may be the explanation, it is unlikely that it is to be found in the relative length of the period of exposure of the *Apanteles* cocoons to the attack of parasites in the two studies, for in both cases every effort was made to obtain the maximum parasitization. The methods of handling the cocoons were identical.

In the cocoons of the second generation the disparity is even greater. Again referring to Muesebeck and Dohanian's table (*8, p. 10*), the results of the first two series of second-generation collections (corresponding to the period of exposure of the present collections) were: Cocoons producing hyperparasites, 43.4 percent; cocoons producing adult *Apanteles*, 14 percent; and cocoons producing neither hyperparasites nor *Apanteles*, 42.6 percent. These percentages are based on 18 individual collections totaling 4,513 cocoons. In table 2 of the present paper the measurable amount of parasitization of *A. melanoscelus* reaches the amazing percentage of 84.4, and this is by no means the maximum, since the exposure of these cocoons was for less than half the time ordinarily experienced under field conditions. However, the percentage given by Muesebeck and Dohanian is based only on the number of cocoons producing adults of the secondaries, whereas the present percentage is obtained from cocoons which in any way were shown to have contained secondary parasites in any stage.

It is of interest to note that the two most abundant species of secondary parasites found by Muesebeck and Dohanian were likewise the most abundant in these experiments.

Parasitization in individual collections of the first-generation cocoons of *Apanteles melanoscelus* ranged from 5.3 percent in a collection of 38 to 70.3 percent in a lot of 37. In the second generation it ranged from 26.6 percent in a collection of 30 cocoons to 100 percent in a group of 149. This last collection was of interest because not a single *Apanteles* adult issued and because 143 of these cocoons produced adults of *Eurytoma appendigaster*, with the possibility that the remaining 6 cocoons which contained dead secondary parasite larvae may have been killed also by this species.

Only one tertiary parasite was reared in the work with *Apanteles melanoscelus*—a single adult of *Pleurotropis tarsalis* (Ashm.) reared from a female pupa of *Eurytoma appendigaster*. This represents only about 0.4 percent parasitization of *Eurytoma*.

APANTELES SOLITARIUS (RATZEBURG)

Among the parasites of the satin moth, one, *Apanteles solitarius*, spends the winter in two ways—as mature larvae in cocoons and as first-instar larvae in the host caterpillars. Very little information is available concerning the parasitization of the overwintering cocoons of this parasite, since they have never been plentiful. Difficulty has been experienced in obtaining summer-formed cocoons also, but the species is increasing quite rapidly, and recently several large collections have been obtained of these cocoons spun mostly by larvae which had hibernated in the host caterpillars.

HIBERNATING COCOONS

The parasitization of *Apanteles solitarius* hibernating in cocoons, as here recorded, refers only to those individuals which spent the preceding winter in the cocoons. The collections were made in the spring, and it was found that because of the weathering of the cocoons it was very difficult to distinguish those which had been formed during the previous fall from those a year older. The method adopted was to collect all cocoons not having exit holes. This probably resulted in a few old cocoons being collected and also in some of the parasitization of the generation in question being missed owing to the hyperparasites completing development and issuing before the advent of cold weather. This was hardly to be avoided, however, for in attempting to collect all the cocoons encountered and to identify the hyperparasites which had already issued, the situation would only have been made worse because of the mixing together of the cocoons formed in two different years.

A total of 44 hibernation cocoons were obtained from eight localities during the two seasons, 1930 and 1932. The results are shown in table 3. One specimen of *Pleurotropis nawaii* (Ashm.) was found dead within a pupa of one of the undetermined secondary parasites.

The parasitization in individual collections ranged from 0 to 100 percent, but the numbers of cocoons in the collections were always very small.

COCOONS OF SUMMER-ISSUING GENERATIONS

Eleven collections were made of cocoons from which the adults would issue during the current season, 1 collection in 1929, 2 in 1930, 1 in 1931, and 7 in 1932. Eight localities were included, one of which furnished a collection in each of the 4 years. Altogether, 1,526 cocoons were obtained and isolated; the results are shown in the second part of table 3. It will be noted that the percentage of cocoons producing adults of *Apanteles* is considerably higher than was the case with the hibernation cocoons, which is to be expected in view of the very much shorter period in which they were exposed to the attacks of hyperparasites. In this connection it should be stated that most of the secondary parasites reared from these cocoons were found to have developed upon pupae, whereas in the case of the hibernation cocoons, and with all the other primary parasites discussed in this paper, the development of the secondaries has been upon the larval or the nymphal stage. The writer believes that this may be explained by the fact that with these summer-issuing generations of *A. solitarius* the period spent in the cocoons is very short

and the development rapid. Thus the hyperparasites on discovering the cocoons only a few days after their formation find the *Apanteles* already in the pupal stage. In the case of the most abundant secondary parasite, *Dibrachys boucheanus*, the individuals in 37, or 22.2 percent, of the cocoons were unable to complete development on the pupae thus encountered. Whether this was due to the continuation of development in the pupae after the eggs were deposited by *Dibrachys* with the consequent diminution in the available food supply for the larvae, or whether it was the result of superparasitism or of other causes is not known. About 3.7 percent of the *D. boucheanus* were killed by *Pleurotropis nawaii* acting as a tertiary in the pupae, one adult issuing from each pupa of *D. boucheanus*. An additional cocoon produced one adult of *D. boucheanus* and one of *P. nawaii*, each developing as a secondary.

TABLE 3.—*Parasitization of Apanteles solitarius, 1929–32*

IN HIBERNATION COCOONS

Species of parasite	Cocoons of *A. solitarius*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Dibrachys boucheanus (Ratzeburg)	4	9.1	4	6	1.5
Undetermined species	8	18.2			
Total killed by parasites	12	27.3			
Adults of *A. solitarius* issued	22	50.0			
Dead from unknown causes	10	22.7			
Total	44	100.0			

IN COCOONS OF SUMMER-ISSUING GENERATIONS

Dibrachys boucheanus (Ratzeburg)	167	10.9	[a] 130	241	1.8
Eupteromalus nidulans (Thomson)	28	1.8	28	28	1.0
Hemiteles tenellus (Say)	9	.6	9	9	1.0
Gelis bucculatricis (Ashmead)	8	.5	8	8	1.0
Gelis apantelis Cushman	7	.4	7	7	1.0
Gelis urbanus (Brues)	4	.3	4	4	1.0
Elasmus atratus Howard	3	.2	3	7	2.3
Eurytoma appendigaster (Swederus)	3	.2	3	3	1.0
Eupelminus saltator (Lindeman)	2	.1	2	2	1.0
Horismenus microgaster (Ashmead)	1	.1	1	1	1.0
Hemiteles fulvipes Gravenhorst	1	.1	1	1	1.0
Pleurotropis nawaii (Ashmead)[b]	1	.1	1	1	1.0
Undetermined species	70	4.6			
Total killed by parasites	304	19.9			
Adults of *A. solitarius* issued	974	63.8			
Dead from unknown causes	248	16.3			
Total	1,526	100.0			

[a] Total number of cocoons giving adults.
[b] Cocoon gave 1 adult of *P. nawaii* and 1 of *D. boucheanus*.

The *Apanteles* in 7 of the cocoons noted in table 3 as having died from unknown causes may have been killed by the fungus *Beauveria ? globulifera* (Speg.) Pic.[4] This is known to be a species capable of acting either as a parasite or as a saprophyte, and although it is considered by some mycologists to be primarily parasitic, there is no evidence in this case to indicate its true status.

[4] Identification and information by M. T. Smulyan, formerly of the Bureau of Entomology laboratory, Melrose Highlands, Mass.

In table 3 *Eupteromalus nidulans* is shown as attacking *Apanteles solitarius* to the extent of 1.8 percent. This is probably due to the fact that *Eupteromalus*, in hibernating in the satin-moth webs, finds these cocoons available very soon after its issuance in the spring. It may pass one or more generations while acting as a parasite of *A. solitarus* before it again turns to its more important role as a primary parasite of the satin moth. In the period covered by these studies, its importance as a parasite of *A. solitarus* is indicated by its attack of 1.8 percent of the summer-issuing cocoons, while its value as a primary parasite of the hibernating satin-moth larvae is shown by its parasitization of 28 percent in all webs examined.

The hyperparasitism in individual collections ranged from 0 in a lot of 18 cocoons to 30.8 percent in a collection of 13.

METEORUS VERSICOLOR (WESMAEL)

AS A BROWN-TAIL MOTH PARASITE

As a parasite of the brown-tail moth *Meteorus versicolor* (*5, pp. 201–205*) spends the winter as a first-instar larva within the partly grown caterpillar of its host. Development is completed in the spring, and the cocoons of *Meteorus* are each suspended by a long thread from the branches of the tree on which the host larvae were feeding. The cocoons are so easily moved about by the wind and the foothold is so unstable that it is strange that the hyperparasites are able to complete oviposition successfully. The process was observed several times in the case of *Hemiteles tenellus*, where, with much beating of wings and repeated short flights when the balance was upset, it was finally completed.

Although *Meteorus* has more than one generation upon the brown-tail moth as host each year, it seems desirable to treat the cocoons of the various generations as a unit, owing to the tendency of the generations to overlap and the consequent difficulty of distinguishing between them, together with the general scarcity of the cocoons in the field and the resulting rather small total collected. As shown in table 4, 357 cocoons were examined. These were obtained in 15 collections from 7 localities over a period of 4 years.

TABLE 4.—*Parasitization of Meteorus versicolor, 1929–32*

Species of parasite	Cocoons of *M. versicolor*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Hemiteles tenellus (Say)	60	16.8	60	60	1.0
Eurytoma appendigaster (Swederus)	28	7.8	28	28	1.0
Cirrospilus cinctithorax (Girault)	3	.8	3	27	9.0
Monodontomerus aereus Walker	2	.6	2	3	1.5
Eupteromalus nidulans (Thomson)	2	.6	2	2	1.0
Dibrachys boucheanus (Ratzeburg)	1	.3	1	1	1.0
Eupelmus spongipartus Foerster	1	.3	1	1	1.0
Gelis urbanus (Brues)	1	.3	1	1	1.0
Habrocytus phycidis Ashmead	1	.3	1	1	1.0
Thysiolorus triangularis (Cresson)	1	.3	1	1	1.0
Undetermined species	8	2.2			
Total killed by parasites	108	30.3			
Adults of *M. versicolor* issued	215	60.2			
Dead from unknown causes	34	9.5			
Total	357	100.0			

In table 5 are given the results of some earlier collections made by various members of the laboratory staff and reared by C. F. W. Muesebeck and S. M. Dohanian. In comparing the figures in tables 4 and 5 it is to be noted that the evident parasitization was greater in the cocoons collected for the present study, but this is not strictly true, for the degree of parasitization expressed in table 5 probably does not represent the whole amount but only that of the cocoons from which adult hyperparasites issued. As a consequence, part of the larger percentage of individuals included in the grouping "Dead from unknown causes" (table 5) may have been killed by secondary parasites. The difference between the percentages of cocoons yielding adults of *Meteorus* in tables 4 and 5 may or may not be significant.

TABLE 5.—*Parasitization of Meteorus versicolor, 1921–22* [a]

Species of parasite	Cocoons of *M. versicolor*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Hemiteles tenellus (Say)	94	14.7	94	94	1.0
Dibrachys boucheanus (Ratzeburg)	[b] 27	4.2	1	3	3.0
Monodontomerus aereus Walker	6	.9	6	8	1.3
Eupelminus sp. [c]	5	.8	5	5	1.0
Gelis sp. [c]	2	.3	2	2	1.0
Habrocytus sp. [c]	1	.2	1	1	1.0
Hemiteles sp. [c]	1	.2	1	1	1.0
Mesochorus sp. [c]	1	.2	1	1	1.0
Undetermined species [c]	12	1.8	4	31	7.8
Total killed by parasites	149	23.3			
Adults of *M. versicolor* issued	341	53.3			
Dead from unknown causes	[d] 150	23.4			
Total	640	100.0			

[a] These collections were made by various members of the laboratory staff. The rearing and identification of the secondary parasites were by C. F. W. Muesebeck and S. M. Dohanian.

[b] Total number of cocoons for which the records are complete as regards the number of secondary parasite adults to issue from each.

[c] The specimens cannot now be located for specific identification.

[d] It is doubtful whether the cocoons from which nothing issued were examined for the presence of dead immature forms. Thus some parasitization is probably included in the category "Dead from unknown causes."

A further comparison of these tables shows that *Hemiteles tenellus* is by far the most abundant secondary parasite in both sets of data, but that the remaining species common to both tables are variable in their relative abundance.

The cocoons which furnished the data for table 5 were obtained in 14 collections from 8 localities during 2 seasons. The parasitization in individual collections ranged from 4.5 percent in a lot of 88 cocoons to 59.2 percent in a lot of 71.

In the present study the parasitization in individual collections ranged from 0 in very small collections to 95.2 percent in a lot of 21, where the *Meteorus* in 20 of the cocoons were killed by secondaries and the remaining cocoon may have been so, as no *Meteorus* issued.

AS A SATIN-MOTH PARASITE

Since a species of *Meteorus* known to be morphologically similar to *M. versicolor* was found by United States Department of Agriculture workers at the Bureau of Entomology laboratory at Budapest,

Hungary, to be a valuable parasite of the satin moth at various points in Europe, many cocoons of this species were collected and sent to this country, where the adults were liberated. Subsequently individuals of what are believed to be this species have been recovered in increasing numbers from both hibernating and summer-feeding satin-moth larvae in New England. C. F. W. Muesebeck, after a careful study of the adults from both the brown-tail moth and the satin moth as hosts, stated [5] that in his opinion they are indistinguishable, although certain details in the biology of those from the satin moth (such as the proportion of sexes) apparently differ from those of the individuals from the brown-tail moth.

Only 9 cocoons have been found and isolated, and of these 6 produced adults of *Meteorus*, 1 an adult of *Hemiteles tenellus*, 1 an adult of *Gelis apantelis;* the *Meteorus* in the remaining cocoon died from an unknown cause. Both hyperparasites were reared from a collection of 5 cocoons made in 1932. It is probable that the degree and nature of the parasitization of *Meteorus versicolor* when it is a parasite of the satin moth will be found to be much the same as when it is a parasite of the brown-tail moth.

HYMENOPTERA, PTEROMALIDAE

EUPTEROMALUS NIDULANS (THOMSON)

As pointed out in a previous paper (*9*), *Eupteromalus nidulans* is of much greater importance as a primary parasite, though capable of acting either as a primary or a secondary. This importance has been more evident in the last few years in its actions as a primary upon both the satin moth (its chief host) and the brown-tail moth. In table 6 it can be seen that the numbers of hibernating *Eupteromalus* found in brown-tail-moth hibernacula have greatly increased since 1928, although the number of hibernacula examined each year has remained about the same (about 250, not taking into account the large collections made for colonization purposes as indicated in a footnote to the table).

TABLE 6.—*Parasitization of Eupteromalus nidulans*

Date of collection, winter of—	E. nidulans collected—		Parasitization by—			
	In webs of brown-tail moth	In webs of satin moth	*Pleurotropis nawaii*		*Pleurotropis tarsalis*	
			Number	*Percent*	*Number*	*Percent*
1926–27	[a] 90					
1927–28	[a] 84		2	2.4		
1928–29	[a] 544					
1929–30	709					
1930–31	959		201	21.0		
1931–32	795		27	3.4		
1930–31 [b]	1,769		441	[c] 24.9		
1931–32 [b]	3,785		982	[c] 25.9	3	0.1
1931–32		56	6	10.7		
Total	8,735	56	1,659	18.9	3	

[a] The notes on some of these earlier collections are incomplete in regard to the amount of parasitization and to the species concerned. The figures given here represent only the opinion of the writer after examining the available data.

[b] The issuance from these collections of the adults of *E. nidulans* and of its parasites was delayed by refrigeration until the middle of the summer of 1931 and 1932, respectively, so that newly issued adults of *E. nidulans* would be produced at the desired time for colonization purposes.

[c] Based on total number of issuing adults of *Eupteromalus* and *Pleurotropis*.

[5] In a letter to the writer, dated Nov. 15, 1932.

In table 6 it will be noted that *E. nidulans* became abundant in the webs collected during the winter of 1928–29, but that the hyperparasite *Pleurotropis nawaii* did not appear in large numbers in the collections until 2 years later, although from its abundance at that time it seems likely that it had been increasing previously and simply had not been obtained in the samples taken. *P. tarsalis* apparently occurs only rarely as a parasite of *Eupteromalus*. The percentage of parasitization by both species of *Pleurotropis* based on the total number of *Eupteromalus* collected in the entire period is 18.9. This percentage is somewhat smaller than those obtained from the large collections of brown-tail-moth webs made for colonization purposes.

Comparatively little is known of the extent of parasitization of *Eupteromalus* when it is a primary parasite of the satin moth. General observations indicate that under these conditions it has always been rather negligible. During the winter of 1931–32 *Pleurotropis nawaii* was found attacking *Eupteromalus* at 3 of the 11 localities where the latter was parasitic upon the satin moth, when the percentage of parasitization was only 10.7 for the area as a whole. At these three points *Eupteromalus* had been abundant in the hibernacula of this host during the previous 2 or 3 years.

DIPTERA, TACHINIDAE

COMPSILURA CONCINNATA MEIGEN

The tachinid *Compsilura concinnata* (*3*) is one of the most important parasites of the gypsy moth, the brown-tail moth, and the satin moth, and in addition it attacks a variety of other hosts of lesser economic importance. Although the puparia of this species are usually formed at or just below the surface of the ground, as with most tachinids, yet when *C. concinnata* is a parasite of the brown-tail moth, the puparia are normally formed near the host itself, the larvae not dropping to the ground. This is true to a lesser extent when the gypsy moth is the host. As a result, a great many more puparia were obtained from the cocoon masses of the brown-tail moth than from any other host.

AS A BROWN-TAIL MOTH PARASITE

A total of 771 puparia of *Compsilura* as a parasite of the brown-tail moth was obtained in 39 collections from 18 localities over a period of 4 years. As indicated by these figures, many of the collections were small, but even among the larger lots the parasitization ranged from 0 in a collection of 28 puparia to practically 100 percent in a lot of 32, where 31 were found to have been killed by *Monodontomerus aereus* and the other died from an unknown cause. The average percentage of parasitization is very high, as shown in table 7, *M. aereus* causing more than two thirds of the total amount. This species has long been known to be an important parasite of *Compsilura*, but to what extent had never been determined until the present study was made. Its increase is made the greater by its ability to mature an average of 7.8 adults to each puparium of its host. Of the 2,047 adults reared, 1,060 were males and 987 females.

Monodontomerus aereus in turn was parasitized by *Pleurotropis nawaii*, 89 individuals, or 4.2 percent of the total, having been reared as against 2,047 adults of *Monodontomerus*.

TABLE 7.—*Parasitization of Compsilura concinnata when it is a parasite of the brown-tail moth, the gypsy moth, and the satin moth, 1929–32*

AS A PARASITE OF THE BROWN-TAIL MOTH

Species of parasite	Puparia of *Compsilura*		Issuance of parasites		
			Host puparia concerned	Total adults issued	Average adults per puparium
	Number	*Percent*	*Number* [a]	*Number*	*Number*
Monodontomerus aereus Walker	324	42.0	264	2,047	7.8
Eurytoma appendigaster (Swederus)	27	3.6	24	24	1.0
Dibrachys boucheanus (Ratzeburg)	14	1.8	12	192	16.0
Brachymeria compsilurae (Crawford)	11	1.4	11	11	1.0
Psychophagus omnivorus (Walker)	5	.6	4	25	6.2
Undetermined species	80	10.4			
Total killed by parasites	461	59.8			
Adults of *Compsilura* issued	196	25.4			
Dead from unknown causes	114	14.8			
Total	771	100.0			

AS A PARASITE OF THE GYPSY MOTH

Dicrachys boucheanus (Ratzeburg)	21	6.8	21	214	10.2
Brachymeria compsilurae (Crawford)	17	5.6	17	17	1.0
Miotropis clisiocampae Ashmead	1	.3			
Undetermined species	11	3.6			
Total killed by parasites	50	16.3			
Adults of *Compsilura* issued	182	59.5			
Dead from unknown causes	74	24.2			
Total	306	100.0			

AS A PARASITE OF THE SATIN MOTH

Brachymeria compsilurae (Crawford)	1	11.1	1	1	1.0
Phygadeuon subfuscus Cresson	1	11.1	1	1	1.0
Dibrachys boucheanus (Ratzeburg)	1	11.1	1	1	1.0
Psychophagus omnivorus (Walker)				1	1.0
Undetermined species	1	11.2			
Total killed by parasites	4	44.5			
Adults of *Compsilura* issued	3	33.3			
Dead from unknown causes	2	22.2			
Total	9	100.0			

[a] Total number of puparia giving secondary parasite adults.

Dibrachys boucheanus was also attacked by *Pleurotropis nawaii*, 192 adults of *Dibrachys* having been reared and 13 individuals of *Pleurotropis*, a parasitization of 6.3 percent.

One adult of *Pleurotropis tarsalis* was reared as a parasite of *Eurytoma appendigaster*, which would represent a parasitization of 4 percent.

AS A GYPSY-MOTH PARASITE

Considerably less is known regarding the parasitization of *Compsilura* when its host is the gypsy moth. The puparia are very difficult to find unless both host and parasite are especially abundant; and even then, in several cases where special efforts were made, none could be found in or on the ground. A total of 306 puparia have been obtained in five collections from as many localities over a period of 3 years. In the second part of table 7 the percentage of parasitization of *Compsilura*

as a gypsy-moth parasite is shown to be considerably lower than was the case when *Compsilura* was parasitic upon the brown-tail moth. While based on a much smaller number of puparia, this percentage is not greatly different from the parasitization of *Compsilura* when a parasite of the brown-tail moth if that part due to *Monodontomerus* is subtracted. In this case the primary lepidopterous host is one not especially subject to the attack of *Monodontomerus*. In fact *Monodontomerus* was not reared at all from these collections, although it is very general in its hyperparasitic habits.

The parasitization in individual collections varied greatly, but that of one large collection totaled 15.5 percent.

A record of the rearing of *Conostigmus* n.sp. from a puparium of *Compsilura* is on file at the Melrose Highlands laboratory, the puparium probably having been obtained from the gypsy moth as host.

AS A SATIN-MOTH PARASITE

As a parasite of the satin moth, *Compsilura* has been abundant at several points in the infested area in New England, but despite considerable effort practically not puparia could be collected at these points. As a result only 9 puparia were obtained in 2 collections from 2 localities, made in different years; the first collection consisting of puparia from the surface of the ground and the second of puparia taken from "spin-ups" (the slight webbing spun by the host larva to hold it to the leaf during the pupal period). The third part of table 7 shows the parasitization in these 9 puparia, and while in no way conclusive, it does indicate that about the same species of hyperparasites, except for *Monodontomerus*, attack *Compsilura* when it is a parasite of the satin moth as when it parasitizes the gypsy moth or the brown-tail moth.

One interesting point is the rearing of an adult of *Dibrachys boucheanus* and one of *Psychophagus omnivorus* from the same puparium, where apparently neither interfered with the development of the other, both species being generally gregarious.

EXPERIMENTAL EFFORTS

Because so few puparia were obtained at infestations of the gypsy moth and the satin moth, newly formed puparia of *Compsilura concinnata* were taken from the laboratory into the field at infestations of these hosts and exposed to the attack of hyperparasites. A total of 680 puparia were exposed in various ways at several infestations. After a period of 1 week careful search resulted in the finding of only 113 puparia intact, the others having been destroyed by an unknown agency. No hyperparasites were reared from any of these 113 puparia.

STURMIA NIDICOLA (TOWNSEND)

The important parasite *Sturmia nidicola* (*6*) is specific upon the brown-tail moth, forming its puparium within the larval skin of the prepupal host larva. It spends the winter as a first-instar larva within the esophagus of its host, from which it migrates when feeding is resumed in the spring. A total of 1,331 puparia were obtained in 31 collections from 17 localities in the New England area infested with its host, from 1929 to 1931, inclusive. Table 8 gives the results obtained from these collections.

TABLE 8.—*Parasitization of Sturmia nidicola, 1929–31*

Species of parasite	Puparia of *S. nidicola*		Issuance of parasites		
			Host puparia concerned	Total adults issued	Average adults per puparium
	Number	*Percent*	*Number*	*Number*	*Number*
Monodontomerus aereus Walker	129	9.7	[a] 122	739	6.0
Brachymeria compsilurae (Crawford)	62	4.7	62	62	1.0
Itoplectis conquisitor (Say)	1	.1	1	1	1.0
Undetermined species	122	9.1			
Total killed by secondary parasites	314	23.6			
Adults of *S. nidicola* issued	527	39.6			
Dead from unknown causes	490	36.8			
Total	1,331	100.0			

[a] Total number of puparia producing secondary parasite adults.

It will be noted that 36.8 percent of all puparia collected were "dead from unknown causes." This high unexplained mortality was evident in all collections and amounted to over half in some. It is not known whether this was produced by the oviposition activities of *Monodontomerus aereus* and other secondaries, but it is suspected that such was the case, at least in part.

Monodontomerus aereus is again the most abundant hyperparasite.

STURMIA SCUTELLATA (ROBINEAU DESVOIDY) [6]

Sturmia scutellata, a large tachinid, is practically a specific parasite of the gypsy moth in New England, laying its eggs on the foliage, where they are eaten by the larvae of about the fourth instar. The maggots, when they have completed development, issue from the pupae of the host and drop to the ground, which they enter to a depth of 2 or 3 inches. They pupate and remain in the ground until the following May or June, when the adults emerge. Parasitization therefore takes place either before the *Sturmia* larvae leave the host or after they have burrowed into the earth. It is known positively that in the case of two species mentioned in table 9 (*Brachymeria* and *Perilampus*) the attack occurs before the maggots of *S. scutellata* have left the host pupae. Such may be the case with *Conostigmus* and *Phygadeuon* also, although this point could not be proved, as the puparia in the collections from which these species issued were allowed to remain in the ground for some time after the maggots had entered or else were kept in containers which hyperparasites might have entered, and hence the attack could have occurred after the puparia were formed.

One thousand three hundred puparia were obtained in 10 collections from 6 localities over a period of 5 years, as shown in table 9. Most of these puparia were obtained by making collections of gypsy-moth pupae from localities in which *Sturmia scutellata* was known to be abundant. These pupae were concentrated around the base of a tree over a piece of wire screening sunk about 1 foot below the surface of the ground. The earth was later sifted for the puparia which had been formed by the larvae thus allowed to leave the hosts and enter the soil in a nearly natural manner. The puparia were then carried

[6] Practically all the work with this species was done by T. H. Jones and assistants, of the Bureau of Entomology, Melrose Highlands, Mass.

through the period of hibernation in soil or in jars with provision for an adequate moisture supply.

TABLE 9.—*Parasitization of Sturmia scutellata, 1927–31*

Species of parasite	Puparia of *S. scutellata*		Issuance of parasites		
			Host puparia concerned	Total adults issued	Average adults per puparium
	Number	*Percent*	*Number*	*Number*	*Number*
Brachymeria compsilurae (Crawford)	121	9.3	[a] 117	117	1.0
(*Megaspilus*) *Conostigmus virginicus* (Ashmead)	24	1.8	24	224	9.3
Perilampus hyalinus Say [b]	1	.1	1	1	1.0
Phygadeuon subfuscus Cresson	1	.1	1	1	1.0
Total killed by parasites	147	11.3			
Adults of *S. scutellata* issued	500	38.5			
Dead from unknown causes	653	50.2			
Total	1,300	100.0			

[a] Total number of puparia giving secondary parasite adults.
[b] Determined by P. B. Dowden, of the Bureau of Entomology laboratory, Melrose Highlands, Mass.

It is seen in table 9 that *Sturmia scutellata* is not extensively parasitized. Probably the figures given do not represent the full amount of parasitization, as no puparia failing to yield adults of *S. scutellata* or its parasites were examined for the cause of death except in 1932. The numbers omitted would add only slightly to the total number killed by parasites, however, since the greater part of those recorded as "dead from unknown causes" may well have been those individuals which failed to hibernate successfully, as it is known that there is considerable mortality from this cause. *Brachymeria compsilurae* is by far the most important parasite of *Sturmia scutellata*.

In table 10 are brought together all the available data concerning the abundance of *Brachymeria* obtained in experiments performed by various members of the laboratory staff. These experiments were carried out for other purposes, and no attention was paid to any hyperparasites other than *B. compsilurae*. The huge total of 24,070 puparia was obtained over a period of 4 years and from many localities, so the percentage yielding *Brachymeria* is very dependable. This is not greatly different from the percentage shown in table 9.

TABLE 10.—*Parasitization of Sturmia scutellata by Brachymeria compsilurae, 1925–27 and 1931*

Item	Puparia of *S. scutellata*	
	Number	*Percent*
Killed by *Brachymeria compsilurae*	1,938	8.1
Adult *S. scutellata* issued	11,586	48.1
Dead from unknown causes	10,546	43.8
Total	24,070	100.0

CARCELIA LAXIFRONS VILLENEUVE

The tachinid *Carcelia laxifrons* (*1, p. 136*) has never been abundant enough to permit the making of large collections, but its puparia are occasionally encountered in the pupal "spin-ups" of the brown-tail

moth. Eighteen puparia were obtained in 4 collections from as many points from 1929 to 1931, inclusive. Eight puparia produced adult *Carcelia*, 2 yielded hyperparasites, and the remaining 8 pupae died from unknown causes. Of the 2 killed by secondaries, 1 produced *Brachymeria compsilurae* and the other was found to contain many dead larvae of *Monodontomerus aereus*.

It is probable that the puparia of *Carcelia laxifrons* will be found to be attacked by the same species of parasites and about as often as are those of *Compsilura* when the latter is acting as a parasite of the brown-tail moth.

Tachina mella Walker

The only native primary parasite discussed in the present paper is *Tachina mella* (*1, p. 112*), the others all having been imported for the control of the gypsy moth, brown-tail moth, satin moth, or oriental moth. Only three puparia of this species have been collected, and in each case it was a parasite of the brown-tail moth; these were found in different years. *T. mella* occurs but rarely as a parasite of the brown-tail moth or at least the puparia are not readily found. Of the 3 puparia collected, 1 produced an adult fly, 1 yielded 6 adults of *Monodontomerus aereus*, and the third pupa died from an unknown cause.

Chaetexorista javana Brauer and Bergenstamm

An important tachinid parasite of the oriental moth has been liberated only recently, but has gained a foothold so quickly that it was possible to obtain 61 puparia in three collections from the infested area of New England in 1932. This tachinid is *Chaetexorista javana*. Every puparium yielded an adult fly.

The puparium of the parasite is formed in the spring within the hibernation cocoon of its host, the latter being so very hard and thick that it is broken only with considerable difficulty. Under these conditions it is not surprising that no hyperparasites were reared. However, 1 of 78 cocoons of the oriental moth collected for another purpose at Jamaica Plain, Mass., in 1930, was found to contain a puparium of *Chaetexorista* which, when examined some time later, contained many dead adults of a species of *Melittobia*. Possibly the attack occurred in the laboratory, as a member of this genus (*M. acasta* Walker) is known to be a laboratory pest (*4, p. 209*), but apparently the present species is able to penetrate the cocoon and puparium to effect oviposition. Perhaps other species may be found to do the same in spite of the nature of the cocoon.

CONCLUSIONS

From these investigations it may be deduced that hyperparasites of the gypsy moth, the brown-tail moth, the oriental moth, and the satin moth play a considerable part in the reduction of the numbers of the primary parasites here considered, a general average of almost one third of the cocoons and puparia being destroyed by them.

In almost every case, each primary parasite has one species of secondary parasite which is far more abundant than any other. In general, this was found to be true in the individual collections and from year to year. Except in the case of the tachinids, where one

might expect the stimulus for oviposition in the puparia of the different species to be much the same, the chief hyperparasitic species was not the same for any two primary parasites.

A summary of the 10 preceding tables is given in table 11 with the inclusion of the corresponding data on *Carcelia laxifrons* and *Tachina mella*.

TABLE 11.—*Summary of parasitization in New England of the primary parasites of the gypsy moth, the brown-tail moth, the satin moth, and the oriental moth*

Primary parasite	Adult primaries issued		Primaries attacked by secondaries		Dead from unknown causes	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Apanteles lacteicolor	840	55.1	76	5.0	608	39.9
Apanteles melanoscelus I	210	51.7	132	32.5	64	15.8
Apanteles melanoscelus II	25	10.0	211	84.4	14	5.6
Apanteles solitarius, winter cocoons	22	50.0	12	27.3	10	22.7
Apanteles solitarius, summer generations	974	63.8	304	19.9	248	16.3
Meteorus versicolor, 1921–22	341	53.3	149	23.3	150	23.4
Meteorus versicolor, 1929–32	215	60.2	108	30.3	34	9.5
Eupteromalus nidulans			1,662	18.9		
Compsilura concinnata on brown-tail moth	196	25.4	461	59.8	114	14.8
Compsilura concinnata on gypsy moth	182	59.5	50	16.3	74	24.2
Compsilura concinnata on satin moth	3	33.3	4	44.5	2	22.2
Sturmia nidicola	527	39.6	314	23.6	490	36.8
Sturmia scutellata, 1927–31	500	38.5	147	11.3	653	50.2
Sturmia scutellata, various records	11,586	48.1	1,938	8.1	10,546	43.8
Carcelia laxifrons	8	44.4	2	11.1	8	44.5
Tachina mella	1	33.3	1	33.3	1	33.4

SUMMARY

Only 5 percent of the cocoons of *Apanteles lacteicolor* were proved to have been killed by secondary parasites, although not all the parasitization was measurable. Adult *Apanteles* issued from 55.1 percent of the total number collected, which is a higher percentage than is found in many of the other parasites. *Eupteromalus nidulans* was the chief hyperparasite.

Parasitization of the cocoons of *Apanteles melanoscelus* was found to be 32.5 percent in the first generation and 84.4 percent in the second. No effort was made to obtain a large collection of the second-generation cocoons, but of the small number received, adult *A. melanoscelus* issued from 10 percent. *Eurytoma appendigaster* was the most abundant parasite of the cocoons of both generations.

Hibernation cocoons of the parasite *Apanteles solitarius* yielded 27 percent of hyperparasites, and those formed during the summer showed 20 percent attack by secondaries. Adults of *A. solitarius* issued from half of the hibernation cocoons and from 64 percent of those of the summer generations. *Dibrachys boucheanus* was by far the most abundant parasite of the cocoons of all generations.

Thirty percent of the cocoons of *Meteorus versicolor* were killed by parasites, and 60 percent yielded adults of *Meteorus*. *Hemiteles tenellus* was the chief parasite.

Of the individuals of *Eupteromalus nidulans* collected from all sources, 19 percent were killed by secondaries, and most of the remainder issued as adults. *Pleurotropis nawaii* was the only secondary parasite of any importance.

The parasitization of *Compsilura concinnata* is presented as found in relation to the three important primary hosts. As a parasite of the

brown-tail moth, *C. concinnata* was attacked chiefly by *Monodontomerus aereus;* as a parasite of the gypsy moth, chiefly by *Dibrachys boucheanus;* and as a parasite of the satin moth, in the few cases found, by several equally.

Parasites killed 23.6 percent of the puparia of *Sturmia nidicola,* and adult *Sturmia* issued from 40 percent. *Monodontomerus aereus* was the most common secondary parasite.

The parasitization of *Sturmia scutellata* was found to be 11 percent, with *Brachymeria compsilurae* responsible for 9 percent of it in the present study and 8 percent when based on a total number of 24,070 puparia obtained in another experiment. Adult *S. scutellata* issued from 38.5 percent and 48 percent of the puparia in the two groups.

Eighteen puparia of *Carcelia laxifrons* collected showed 2 (11 percent) parasitized, 1 each by *Brachymeria compsilurae* and *Monodontomerus aereus.* Adult *Carcelia* emerged from 8 of the remaining.

Three puparia of *Tachina mella* yielded 1 adult fly, 1 killed by *Monodontomerus aereus,* and 1 dead from an unknown cause.

No parasitization was found in the puparia of *Chaetexorista javana,* although a single puparium obtained in another experiment contained adults of a species of *Melittobia.*

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THE RELATION OF "DARK CENTER" TO THE COMPOSITION OF RUTABAGAS[1]

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INTRODUCTION

"Dark center" of rutabaga (*Brassica campestris* L.) is a well-known disorder that occurs in New England, Canada, and portions of Europe and New Zealand. It has become so prevalent in certain sections of Massachusetts as to destroy the marketability of a large percentage of the rutabaga crop.

Dark center is characterized by irregular dark streaks in the parenchyma of the rutabaga, with the formation of more or less stringy fibers, and in some instances a water-soaked area; hence the names "mottled heart", "water core", etc. These changes develop rapidly about the time of harvesting, but have not been correlated with any fungous or bacterial disease so far as noted. W. H. Davis, of Massachusetts State College, made cultures on agar and found the tissue sterile. He did find, however, by microscopical examination, a break-down of the cell walls similar to edema, which would seem to indicate a functional disturbance due to environmental conditions.

ANALYSES

Bushel samples of supposedly normal and affected Purple Top rutabagas were drawn from a field in North Eastham, Mass., in 1932 and submitted to the laboratory for analysis. The roots averaged 922 g in weight. Of the 28 apparently normal roots, 16 showed dark streaks when halved vertically, and these were excluded. The 31 affected rutabagas contained 1 marketable root, which was likewise excluded. Neither fibrous formations nor water-soaked areas were observed in the 30 specimens retained. These are probably symptoms of a more advanced stage of the disorder.

The selected rutabagas were sliced, dried quickly, ground to pass a 1-mm sieve, and produced satisfactory yellowish meals of similar appearance except that the meal from the affected roots was slightly darker. The two samples were analyzed by the ordinary methods. The results are shown in table 1.

As compared with averages published in various compilations, the normal rutabagas were high in carbohydrates (nitrogen-free extract), especially total sugars, and correspondingly low in protein, fiber, and ash. They were of excellent quality as a table product. The roots affected by dark center suffered a substantial loss in nitrogen-free extract, with an increase in protein, fiber, and ash. The loss in total sugars was partly compensated by gains in pentosans and galactan (gums).

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[2] The writers are indebted to G. B. Snyder and R. W. Donaldson, of the Massachusetts State College, for data relative to the dark-center disorder and its distribution.

TABLE 1.—*Analyses (percent) of Purple Top rutabagas when normal and when affected with dark center*

[Results on dry-matter basis]

Constituent	Normal	Affected with dark center	Constituent	Normal	Affected with dark center
Original moisture	89.70	88.72	Nitrogen-free extract—Continued.		
Crude protein (N×6.25)	5.94	9.21	Pentosans	7.39	9.89
Total nitrogen	.95	1.47	Galactan	2.79	6.03
Amino nitrogen (Van Slyke)	.11	.18	Crude fiber (cellulose)	7.28	10.85
Nitrate nitrogen	(a)	(a)	Crude ash	4.07	5.63
Crude fat (ether extract)	1.31	1.20	Acid-soluble ash	4.04	5.63
Nitrogen-free extract	81.40	73.11	Acid-insoluble ash	.03	.00
Dextrose	48.53	36.99	pH at 25° C	5.75	5.63
Sucrose	12.17	10.87			
Residual carbohydrates as starch [b]	7.47	7.90			

[a] None. [b] Little, if any, true starch present.

The initial effect of dark center is the dissipation of an appreciable proportion of the sugars, but to what purpose it is at present impossible to state.

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